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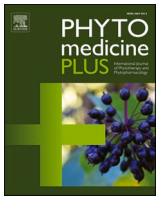

Oral administration of quercetin and fisetin potentiates photocarcinogenesis in UVR-exposed hairless mice

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ABSTRACT

Background: Phytochemicals have demonstrated great potential as photoprotectants. Apple-derived compounds such as quercetin, fisetin, and rutin are reported to provide topical photoprotection, but oral delivery has not been explored. Purpose: To determine the photoprotective effects of oral administration of quercetin, fisetin, and rutin, and their accumulation in skin assessed through mass spectrometry imaging. Study design: Groups of 25 hairless mice (n = 125 mice) received in the daily feed 100 mg/kg quercetin, fisetin, or rutin, 600 mg/kg nicotinamide in water as a positive control, or no supplementation as the UV control. The animals were exposed to ultraviolet radiation (UVR) equivalent to 3.5 standard erythema doses thrice weekly. Method: Mass spectroemetry imaging was used to assess local skin accumulation. Results: Oral administration of quercetin and fisetin reduced the time to tumour onset (Quercetin: second and third tumour [$p < 0.045$]; fisetin: third tumour [$p < 0.021$]), with no observed effect for rutin. Nicotinamide delayed the onset of all three recorded tumours ($p < 0.0082$). Results were supported by accelerated tumour growth following quercetin treatment ($p < 0.0069$), whereas nicotinamide reduced tumour growth ($p < 0.00015$). Skin accumulation of the compounds could not be demonstrated, suggesting other mechanisms must be explored to explain these effects on UVR-induced carcinogenesis. Conclusion: Oral administration of quercetin and fisetin to hairless mice increased UVR-induced tumour development. These results indicate a need for caution when selecting candidates for photoprotectants.

Introduction

Ultraviolet radiation (UVR) is the primary risk factor for keratinocyte carcinomas such as basal cell carcinoma and squamous cell carcinoma (SCC) (Kim, and He, 2014). UVR is classified into UVC (100–280 nm), UVB (280–315 nm), and UVA (315–400 nm) (Dale Wilson et al., 2012). The entirety of UVC and part of UVB are absorbed by the ozone layer, leaving the constituents of UVR affecting the skin UVA (~95 %) and UVB (~5 %) (Slominski, and Pawelek, 1998; El Ghissassi et al., 2009). While UVA penetrates deeper into the skin, UVB is absorbed in the epidermis where its contribution to carcinogenesis is much more potent, in part due to its increased mutagenic potential (Drobetsky et al., 1995).

Despite increasing keratinocyte carcinoma incidence rates and massive sun protection campaigns (Birch-Johansen et al., 2010; Rogers et al., 2015), adherence to proper sunscreen administration remains poor (Holman et al., 2015). To circumvent this limitation, oral delivery of photoprotective compounds has emerged as a promising alternative (Parrado et al., 2018).

Because of their antioxidant activity, phytochemicals have been studied extensively for their potential as anticarcinogenic compounds (Pihl et al., 2021). Quercetin is a flavonol ubiquitously found in natural products such as apples, asparagus, green tea, lettuce, and onions (Nishimuro et al., 2015). Quercetin is reported to act anticarcinogenic in various cancer cell lines such as breast (Choi et al., 2001), lung (Zheng

Abbreviations: DAN, 1,5-Diaminonaphthalene; DHB, 2,5-Dihydroxybenzoic acid; SCC, squamous cell carcinoma; UVR, ultraviolet radiation.

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et al., 2012), and prostate cancer (Nair et al., 2004) due to its anti-proliferative properties (Srivastava et al., 2016). Topical application of quercetin protects against oxidative stress (Casagrande et al., 2006) and inflammation in UV-irradiated hairless mice (Vale et al., 2021). However, oral administration was found to have no effect on carcinogenesis through 17 weeks of UVR (Steerenberg et al., 1997).

To elucidate the protective properties of quercetin on long-term UVR, and to investigate how structurally related compounds differ in photoprotective potential, fisetin and the quercetin glycoside rutin were also tested. Topical application of both rutin and fisetin has demonstrated protection against UVR-induced inflammation in hairless mice through their suppression of MAPK-mediated expression of cyclooxygenase-2 and inducible nitric oxide synthase (Choi et al., 2014; Wu et al., 2017), indicating a similar photoprotective potential for the three compounds.

The present study evaluated the abilities of oral administration of quercetin, fisetin, and rutin to protect against UVR-induced carcinogenesis in hairless mice. We aimed to determine the effects of oral administration of the compounds on UVR-induced tumour onset and development. The compounds' photoprotective effects on measures such as erythema, pigmentation, oedema formation, and epidermal thickness were also investigated. In addition, local accumulation of the compounds in the skin was explored through mass spectrometry imaging.

Materials and methods

Animals

A total of 125 female C3.Cg-*Hr*^{hr}/TifBomTac hairless mice (Taconic, Ry, Denmark) ages 14 to 27 weeks were randomised into groups of 25 animals and used in this study. Animal facilities and handling have been described previously (Pihl et al., 2023). The study protocol was approved by the Danish Animal Experiments Inspectorate (permit number: 2021–15–0201–00,905) and the local animal welfare committee at Bispebjerg Hospital, Denmark.

Compound administration and UVR protocol

The three experimental groups received standard tap water and Altromin 1320 feed modified with either quercetin, fisetin, or rutin to ensure a daily dose of 100 mg/kg (Altromin Spezialfutter GmbH & Co. KG, Lage, Germany). To distinguish between feeds, colourants were added for the first three months of the study (see Table 1 for compounds), whereafter it was removed during the last six months due to excessive colouring in faeces and urine. Based on previous experience (Pihl et al., 2023), nicotinamide (Vitamin B_3; 600 mg/kg) was included as a positive control alongside a non-supplemented control (UV control). The UVR source consisted of five Bellarium-S SA-1–12 tubes (Wolff Systems, Georgia, United States of America) and a single UV6 tube (Waldmann Lighting, Illinois, United States of America). UVB emission (280–315 nm) comprised 5.9 % of the output, which was measured routinely using a spectroradiometer. Animals were exposed to 3.5 standard erythema doses of UVR three times per week. To analyse skin accumulation of the compounds, a 4 mm punch biopsy was collected from anaesthetised animals after two months of UVR and frozen.

Table 1
Treatments for the five study groups.

Group	Feed	Water	Daily compound dose (mg/kg bodyweight)
Fisetin	Modified 1320 feed: 625 mg/kg fisetin, Tartrazine*	Standard tap water	100 mg/kg
Quercetin	Modified 1320 feed: 625 mg/kg quercetin, Allura Red AC*	Standard tap water	100 mg/kg
Rutin	Modified 1320 feed: 625 mg/kg rutin, Patent Blue V*	Standard tap water	100 mg/kg
Nicotinamide	Standard 1320 feed	3.37 g/L nicotinamide in tap water	600 mg/kg
UV control	Standard 1320 feed	Standard tap water	N/A

* Compound removed after three months.

Tumour onset and development

Tumour onset was evaluated weekly. In brief, tumours with a diameter of 1 mm or above were recorded. Size, location, and number of tumours were mapped for all animals, and tumours reaching 4 mm in diameter counted towards estimations of tumour onset (Wulf et al., 2021; Pinto et al., 2022). Tumour onset was recorded for the first three tumours reaching 4 mm. Tumour development was assessed based on tumour number, size, and growth of all tumours with a diameter of 4 mm or above. Tumour size was measured as tumour area estimated using the formula: $\text{tumour area} = \pi \cdot \left(\frac{\text{tumour length}}{2}\right) \cdot \left(\frac{\text{tumour width}}{2}\right)$ (Pihl et al., 2023). Tumour growth was measured as an accumulation of tumours with a diameter of 4 mm or above in each week and determined by the area under the curves for the given treatments (Supplementary Figure 1).

Endpoints and oedema formation through ear punch weight

Mice were euthanised 24 h after the last UVR dose after reaching an endpoint. Endpoints included one tumour with a diameter of 12 mm, three tumours of 4 mm, or at the end of the study period of 365 days. UVR-induced oedema was measured by ear punch weight as described previously (Pihl et al., 2023). To analyse skin accumulation of the compounds, a 4 mm punch biopsy of dorsal skin was collected and frozen.

Erythema

Dorsal erythema was measured weekly during the first six weeks of UVR on a scale from 0 (no erythema) to 4 (very severe erythema) as described previously (Pihl et al., 2023).

Pigmentation and weight

Evaluation of pigmentation and weight of the mice were performed monthly. Following the protocol of Hansen et al. (Hansen et al., 1995), dorsal pigmentation was measured under six Philips TL08 fluorescent UVA tubes (Philips, Eindhoven, The Netherlands) through comparison with a Kodak Gray Scale.

Mass spectrometry imaging

Punch biopsies of 4 mm from the five groups were collected after 2 months of treatment and again at the end of the study period. The biopsies were embedded in a hydroxypropyl-methylcellulose and polyvinylpyrrolidone gel in a chamber set to -20 °C (Dannhorn et al., 2020). Cross sections of the biopsies were cut at 10 µm thickness on a Leica CM3050S cryo-microtome (Leica Microsystems, Wetzlar, Germany), mounted onto slides, and stored at -80 °C.

Frozen sections were thawed in a vacuum desiccator. Sections from quercetin, fisetin, rutin, or UV control mice were coated with a matrix solution of 3.3 mg/mL 1,5-Diaminonaphthalene (DAN) in methanol: water (9:1) with a pneumatic sprayer with a flow rate of 50 µL/min, rotating at 600 RPM for imaging in negative mode. Nicotinamide-

treated sections were coated with 30 mg/mL 2,5-Dihydroxybenzoic acid (DHB) matrix solution in methanol:water (9:1) + 1 % trifluoroacetic acid using an iMatrixSpray for imaging in positive mode (Stoeckli et al., 2014). The spray settings consisted of 12 spray cycles with a height of 80, a line distance of 1 mm, a speed of 90 mm/s, and a density of 2 μL/cm^2.

MALDI imaging was performed on a Thermo QExactive Orbitrap mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany) equipped with an AP-SMALDI5 Ion source (TransMIT GmbH, Giessen, Germany) (Traberg et al., 2022). Imaging of sections covered with the DAN matrix was performed in negative ionisation mode with a scan range of 200–1000 *m/z*. Analysis of nicotinamide-treated sections was performed in positive ionisation mode with a scan range of 100–700 *m/z*. All analyses were acquired with a mass resolving power of 140,000 at *m/z* 200 and a pixel size of 20 μm. The spectra were internally calibrated to the signal of the respective matrix, ensuring a mass accuracy of 4 ppm. Mass spectrometry images were generated and analysed with MSiReader (v1.02) (Bokhart et al., 2018) and all images were pre-processed by normalisation to Total Ion Current (TIC).

To assess the signal of the experimental compounds, one spot (1 μL) of each solution of fisetin (F), quercetin (Q), and rutin (R) (1 mg/mL methanol) was applied to the surroundings of the UV control section. The position of each solution is demonstrated in Fig. 1.

Statistics

Visualisation of tumour development was presented by Kaplan-Meier plots (Kassambara et al., 2021) and analyses of comparisons to the UV control performed using the log-rank test (Mantel-Cox). Individual comparisons to the control were assessed by a non-parametric Mann-Whitney U test performed in R (v4.1.0) (R Core Team, 2021). $p < 0.05$ was considered significant.

Results

UVR-induced tumour onset

Ultraviolet radiation induced tumours in all animals (Fig. 2). Compared to the UV control group, oral administration of quercetin and fisetin reduced the time until the second and third tumour ($p < 0.045$). Mice treated with quercetin had a median of 183 days until the onset of both the second and third tumour compared to the respective 193 and 203 days of the UV control (Fig. 2,Table 2). While both fisetin treatment and the UV control had medians of 203 days until the third tumour, the confidence interval was significantly lower for fisetin-treated mice (Fig. 2, Table 2). No effect was observed following rutin treatment.

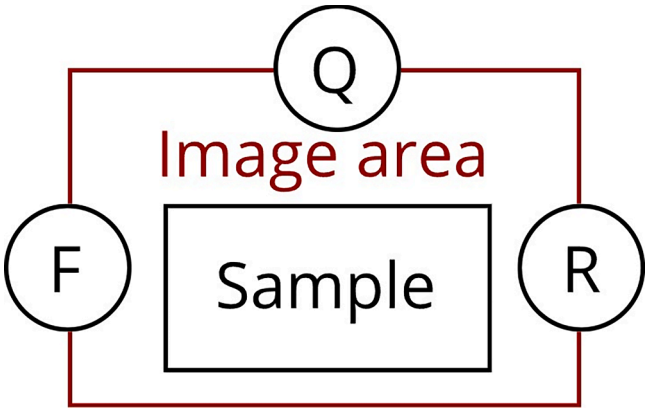

Fig. 1. Schematic of UV control section spiked with phytochemical compounds. UV control sections were analysed with a spot (1 mg/mL methanol) of fisetin (F), quercetin (Q), and rutin (R) applied to the surrounding image area.

Consistent with earlier reports (Pihl et al., 2023), oral nicotinamide administration delayed the onset of all three tumours compared to the UV control (Fig. 2, Table 2; $p < 0.0082$). No weight loss was observed in phytochemical-treated mice indicating no toxicity related to the treatments, but oral fisetin and quercetin did increase weight gain over the study period compared to the UV control group (Supplementary Figure 2; $p < 0.0032$).

Tumour development

Oral administration of the phytochemicals did not influence the number or size of tumours developed (Figs. 3a and b). However, administration of quercetin significantly accelerated tumour growth demonstrated by an increased area under the curve compared to the UV control (Figs. 3c and Supplementary Figure 1, Table 3). Nicotinamide treatment reduced the tumour size from a median of 68.33 mm^2 of the control to 49.48 mm^2 (Fig. 3b, Table 3), as well as tumour growth (Figs. 3c and Supplementary Figure 1), consistent with its protective effect on tumour onset.

Assessment of UVR-induced erythema, pigmentation, and oedema formation

To determine the photoprotective effects of the phytochemicals, erythema, pigmentation, oedema formation, and epidermal thickness were evaluated. Dorsal erythema and pigmentation were apparent in all groups in response to UVR. No difference in accumulated erythema scores was demonstrated following six weeks of exposure to UVR (Fig. 4a). Oral administration of all compounds but rutin increased dorsal pigmentation after 27 weeks of UVR ($p < 0.0325$). UV control mice had a median pigmentation score of 3, which was increased to 4, 4, and 5 by fisetin, quercetin, and nicotinamide, respectively (Fig. 4b, Table 4). To assess long-term UVR-induced oedema, ear punch weights were measured immediately after euthanasia. UVR control mice had a median ear punch weight of 3.53 mg, which was increased to 3.70 mg by fisetin administration (Fig. 4c, Table 4). Histological changes of the skin induced by UVR were measured by epidermal thickness. Representative skin sections stained with haematoxylin and eosin are shown in Supplementary Figure 3. No effect on epidermal thickness was determined across the groups (Table 4).

Imaging-assessed compound accumulation

To assess whether the compounds accumulate locally in the skin, mass spectrometry imaging was performed on cryo-sections of skin biopsies. In addition to the biopsy cryo-sections, sections from a homogenate of mouse skin were spotted with the compounds to give an indication of the detection limit in the skin (see Supplementary materials and Supplementary Figure 4).

The phytochemical compounds were given to the animals through their feed ensuring a daily intake of 100 mg/kg. The complete study period lasted nine months, of which feed colourants were used during the first three months to differentiate between experimental feeds. Fig. 5 shows imaging analysis performed on biopsies taken after two months of UVR exposure. No significant trace of Allura Red and Tartrazine were detected in the skin biopsies from quercetin- and fisetin-fed mice, respectively (Fig. 5). Trace amounts of Patent Blue V were found in skin biopsies from the rutin-treated group (Fig. 5).

Mass spectrometry imaging of the phytochemical compounds was performed on biopsies collected at the end of the study period. The phytochemicals and nicotinamide were not detected in their respective sections (Figs. 6 and 7, respectively). Related metabolites of the phytochemical compounds were also analysed but none were detected (Fig. 6) (See Supplementary Figure 5 and Supplementary Table 1 for an extended panel of metabolites). Following up on the fact that only a few of the administered compounds were detected, limits of detection (in

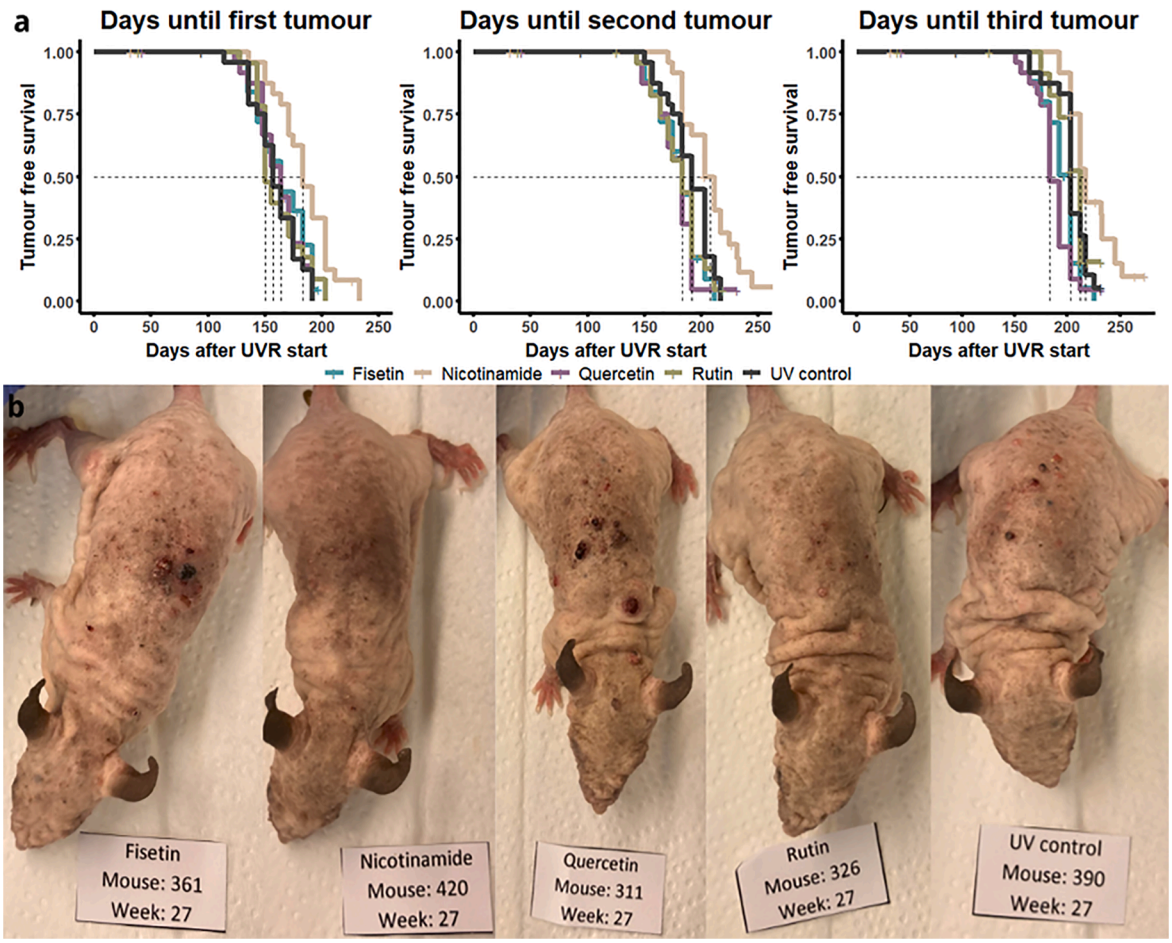

Fig. 2. UVR-induced tumour onset. a: Kaplan-Meier plots of tumour free survival without one, two, or three tumours with a diameter of 4 mm or above. b: representative pictures of early tumour development (1–3 mm) after 27 weeks of UVR.

Table 2
Tumour onset following oral administration of phytochemicals.

Tumour onset		Fisetin	Nicotinamide	Quercetin	Rutin	UV control
1st tumour	Median days	164	183	164	150	157
	95 % CI	150–183	175–203	156–175	150–175	150–175
	p-value	0.326	$2.4 \cdot 10^{-4}$	0.842	0.761	
2nd tumour	Median days	183	207.5	183	183	192
	95 % CI	175–192	203–225	171–192	171–192	183–203
	p-value	0.078	$8.2 \cdot 10^{-3}$	0.045	0.236	
3rd tumour	Median days	203	217	183	212	203
	95 % CI	192–203	212–245	183–192	203–212	203–217
	p-value	0.021	$5.2 \cdot 10^{-3}$	$1.5 \cdot 10^{-3}$	0.778	

μg/g tissue) were established by deposition of standards of the compounds on skin homogenate cryo-sections. The results of this experiment are presented in Supplementary materials.

Discussion

Oral administration of phytochemicals is considered a promising avenue to increase photoprotection. However, for the first time, we show that oral administration of the apple-derived compounds quercetin and fisetin increases ultraviolet radiation-induced carcinogenesis in hairless mice. Quercetin and fisetin reduced the time until tumour onset (Fig. 2 and Table 2), which was supported by quercetin treatment further accelerating tumour growth (Fig. 3c and Table 3). These results could not be explained by local skin accumulation (Figs. 5–7), suggesting other mechanisms must be explored to explain the compounds' effect on UVR-induced carcinogenesis.

As flavonols, the compounds are reported to possess numerous protective properties, most notably an effective antioxidant capacity (Yao et al., 2008; Gęgotek et al., 2019; Rajnochová Svobodová et al., 2022). Topical application of fisetin, quercetin, and rutin has all been shown to reduce the damaging effects of UVR due to antioxidant or anti-inflammatory properties (Casagrande et al., 2006; Vale et al., 2021; Choi et al., 2014; Wu et al., 2017). One study showed that oral administration of quercetin protected against UVR-induced immunosuppression but had no effect on tumour growth in hairless mice through 17 weeks of UVR (Steerenberg et al., 1997).

Despite these reports, we found a reduction in time until tumour onset following oral administration of quercetin and fisetin (Fig. 2), associated with accelerated tumour growth (Fig. 3c) and oedema formation (Fig. 4c), respectively.

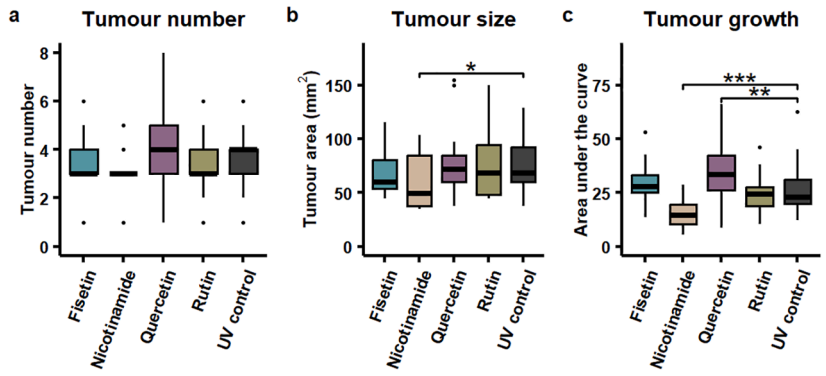

Fig. 3. UVR-induced tumour development. a: number of tumours, b: tumour size, and c: tumour growth expressed as the area under the curve of Figure S1. *, **, and *** signify *p*-values lower than 0.05, 0.01, and 0.001.

Table 3
Evaluation of photocarcinogenesis following oral administration.

		Fisetin	Nicotinamide	Quercetin	Rutin	UV control
Tumour number	Median	3	3	4	3	4
	p-value	0.572	0.092	0.359	0.123	
Tumour size	Median	59.69 mm^2	49.48 mm^2	72.26 mm^2	68.33 mm^2	68.33 mm^2
	p-value	0.159	0.013	0.895	0.886	
Tumour growth	Median	28	14.5	33.5	24.25	23
	p-value	0.129	$1.5 \cdot 10^{-4}$	$6.9 \cdot 10^{-3}$	0.562	

Summary of photocarcinogenesis measures following oral administration consisting of tumour number, tumour size, and tumour growth.

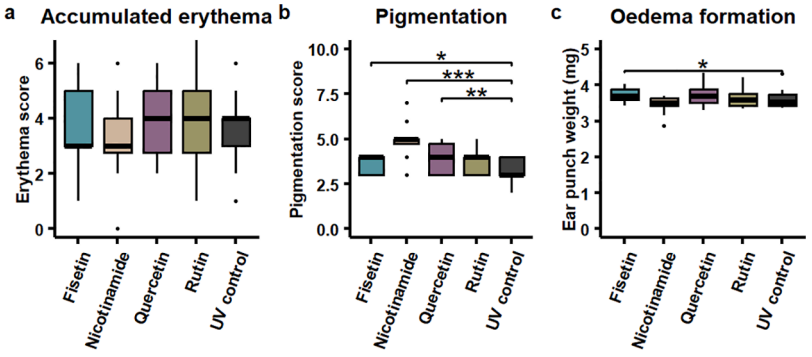

Fig. 4. UVR-induced erythema, pigmentation, and oedema induced. a: accumulation of erythema through the first six weeks of UVR. b: dorsal pigmentation after 27 weeks of UVR. c: oedema formation at the end of the study period. *, **, and **** signify *p*-values lower than 0.05, 0.01, and 0.0001.

Table 4
Evaluation of photoprotective effects demonstrated by the phytochemicals.

		Fisetin	Nicotinamide	Quercetin	Rutin	UV control
Erythema	Median	3.00	3.00	4.00	4.00	4.00
	p-value	0.86	0.54	0.59	0.55	
Pigmentation	Median	4	5	4	4	3
	p-value	0.0325	$3.3 \cdot 10^{-7}$	0.0039	0.1060	
Ear punch weight	Median	3.70 mg	3.52 mg	3.70 mg	3.58 mg	3.53 mg
	p-value	0.0102	0.362	0.131	0.609	
Epidermal thickness	Median	63.40 µm	75.21 µm	77.34 µm	89.22 µm	93.68 µm
	p-value	0.15	0.69	0.15	0.31	

Summary of photoprotective effects following oral administration consisting of erythema, pigmentation, oedema formation, and epidermal thickness.

Both quercetin and fisetin were found to act mutagenic in the *in vitro* Ames *Salmonella typhimurium* assay (Brown et al., 1977; MacGregor, and Jurd, 1978; Brown, 1980). In contrast, rutin was found to exhibit no mutagenic potential, consistent with the results of this study. Quercetin has also been shown to act phototoxic in vitro, inducing an increase in reactive oxygen species (Rajnochová Svobodová et al., 2017). Furthermore, several studies have reported quercetin to be carcinogenic in numerous tissues (Matsukawa et al., 2002; Singh et al., 2010). In particular, a study of azoxymethane-induced adenocarcinomas in the colons of rats identified quercetin as a co-carcinogen (Pereira et al., 1996). Quercetin may therefore potentiate the carcinogenic potential of UVR. Because of their structural similarities and shared mutagenic

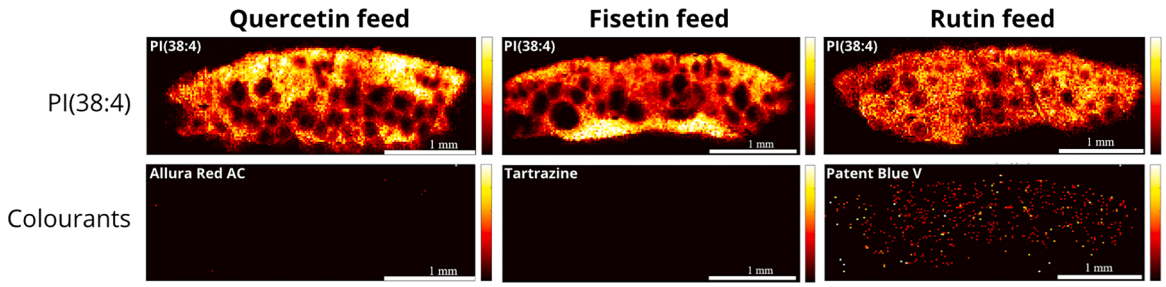

Fig. 5. Mass spectrometry images of colourants in skin cryo-sections from the different groups. Top row: a skin biomarker - phosphatidylinositol (PI) (38:4) detected at *m/z* 885.5493 $[M\text{-}H]^-$. Bottom row: feed colourants from left to right Allura Red at *m/z* 451.02698 $[M\text{-}2Na+H]^-$, Tartrazine at *m/z* 466.9967 $[M\text{-}3Na+2H]^-$, and Patent Blue V at *m/z* 559.15727 $[M\text{-}Na]^-$. The colour scale indicates the relative intensities in the images.

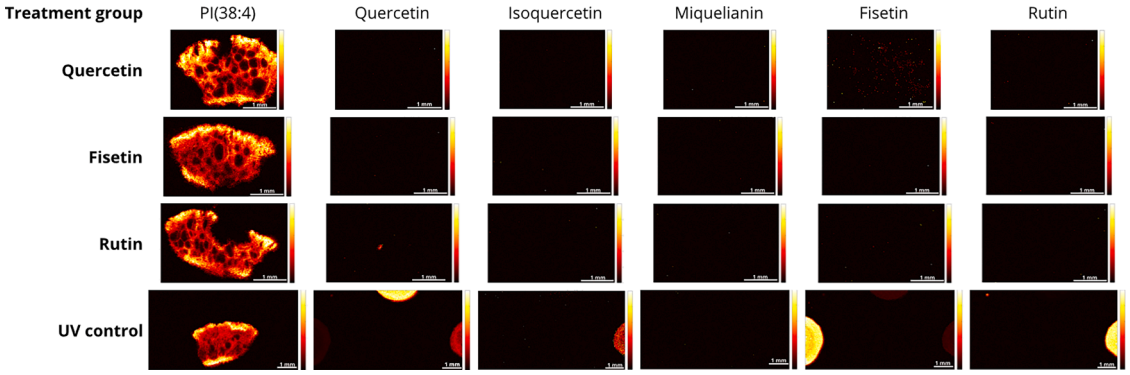

Fig. 6. Mass spectrometry images of phytochemicals and their metabolites in skin cryo-sections from the different groups. Mass spectrometry imaging of phosphatidylinositol (PI) (38:4) signal detected at *m/z* 885.5493 used as a skin biomarker, and experimental compounds and metabolites detected at *m/z* 301.0348 (quercetin), 463.0876 (isoquercetin), 477.0669 (miquelianin), 609.1456 (rutin), and 285.0399 (fisetin). In the UV control section, one droplet (1 mg/mL) of each solution of fisetin (left), quercetin (top) and rutin (right) was deposited to the area surrounding the section. All images were generated for the deprotonated form $[M\text{-}H]^-$. The colour scale indicates the relative intensities in the images.

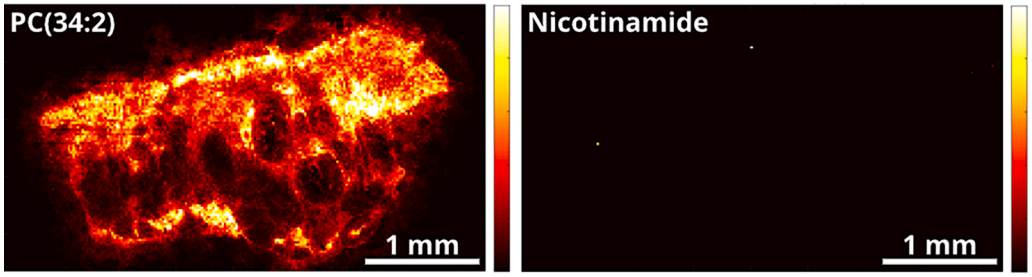

Fig. 7. Mass spectrometry image of nicotinamide in skin cyrosections. Mass spectrometry imaging of phosphatidylcholine (PC) (34:2) $[M+K]^+$ detected at *m/z* 796.5253 used as a skin biomarker, and nicotinamide $[M+H]^+$ detected at *m/z* 123.0553. The colour scale indicates the relative intensities in the images.

potential, it is possible that fisetin also acts as a co-carcinogen.

A similar situation is demonstrated with the phytochemical capsaicin. Capsaicin exhibits numerous anticancer properties (Clark, and Lee, 2016) and has been shown to reduce the metastatic burden in transgenic adenocarcinomas of murine prostates (Venier et al., 2015). But akin to quercetin, capsaicin was found to act as a co-carcinogen on 12-O-tetradecanoylphorbol-13-acetate-induced skin carcinogenesis in mice (Hwang et al., 2010).

It is important to note that rutin is a glycoside consisting of rutinose and quercetin, illustrated by the mass spectrometry imaging detection of quercetin in the area of the UV control section where rutin was applied (Fig. 6). Upon entry into the gut, rutin is metabolised into quercetin (Riva et al., 2020), suggesting that quercetin and rutin administration should result in similar effects. Although differential treatment outcomes for quercetin and rutin have been reported (Domitrović et al., 2012; Lee et al., 2016), the difference in effect could be caused by the concentrations used. If the molecular weights are considered (quercetin: 302.236 g/mol; rutin: 610.57 g/mol), processing of rutin at the given treatment dose would result in half the amount of quercetin released. It is therefore possible that rutin given at an equimolar dose to quercetin would result in a similar effect.

To distinguish between the experimental feeds, colourants were added to the feed pellets along with the phytochemical compounds. We were made aware of reported suspicions that the compounds may induce genotoxicity (Tsuda et al., 2001) and asthma-like symptoms (Juhlin et al., 1972). While no symptoms were observed in our mice, the use of feed colourants was terminated after the first three months and was not used during the last six months of the study. Through mass spectrometry imaging, we could not detect trace amounts of either Allura Red AC or Tartrazine, the colourants added to the quercetin and fisetin feed, respectively (Fig. 5), suggesting that the colourants did not accumulate in the skin. However, accumulation of quercetin and fisetin was also not

found in the skin (Fig. 6) and therefore does not rule out that the colourants may have impacted the carcinogenic effect.

The use of feed colourants has been extensively reviewed by the European Food Safety Authority (EFSA). In their 2021 report (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) et al., 2021), the EFSA state that feeds containing Allura Red AC up to 2000 mg per kg bodyweight do not induce genotoxicity in mice (Honma, 2015). Furthermore, the use of 8336 mg Allura Red AC per kg bodyweight does not induce adverse effects in female mice other than colouration of urine and faeces, as observed in the present study. For chronic feedings like the current study, the EFSA reported that up to 1.39 % of Allura Red AC in the feed resulted in no adverse effect in mice (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) et al., 2021).

Moreso, a recent human study reported the use of Allura Red AC as a control treatment compared to the addition of the carotenoid lutein (Zmitek et al., 2020). The treatments were ingested daily for three months, similar to the current study. No phototoxic effects were observed in the Allura Red AC treated group following UVR, indicating that oral intake of Allura Red AC over a period of three months in combination with ultraviolet radiation is not harmful to the skin.

Given that the feed colourants were used only for the first three out of nine months of the study, at a feed concentration of 0.1 %, the authors believe that quercetin and fisetin themselves must have contributed to the observed acceleration of carcinogenesis. However, the potential impact of the feed colourants must be taken into account when considering the results.

It should be emphasised that while we did not observe any effect on photocarcinogenesis following rutin treatment, we were able to detect trace amounts of the colourant Patent Blue V in biopsies of skin from mice fed the compound for three months (Fig. 5). Although it did not affect the results of our study, the accumulation of colourants in tissue following their addition to feed may impact other outcomes.

Oral quercetin and fisetin treatment increased pigmentation in the present study (Fig. 4b), consistent with the melanogenic properties previously shown for quercetin (Nagata et al., 2004). Dorsal pigmentation is thought to protect against UV radiation by absorbing UV rays (Kobayashi et al., 1998). In a previous study, we found that oral nicotinamide led to protection against photocarcinogenesis associated with increased pigmentation (Pihl et al., 2023), similar to what we report here (Figs. 2 and 4b). However, the study also reported two groups exhibiting a delay in tumour onset without increased pigmentation (Pihl et al., 2023). Topical application of quercetin is shown to protect against UVR-induced DNA damage in artificial skin mimics, but this was attributed to a sunscreen effect (Maini et al., 2015). It is therefore possible that quercetin and fisetin's mutagenic potentials act independently of their abilities to increase pigmentation. As both compounds are given orally, their mutagenicity may continue to augment the carcinogenic environment facilitated by UVR

In summary, we have for the first time shown that oral administration of quercetin and fisetin, but not rutin, increases UVR-induced carcinogenesis, supported by quercetin's acceleration of tumour growth. No local accumulation of the compounds was found in the skin, suggesting there is more to learn about the compounds' mechanisms of action. The potential impact of the feed colourants should be heeded when interpreting the results. Overall, this highlights potential co-carcinogenic properties of quercetin and fisetin when used in combination with ultraviolet radiation that should be carefully considered with future use.

Data availability

Data will be made available upon reasonable request.

CRediT authorship contribution statement

Celina Pihl: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Jonatan Riber Granborg:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Fernanda Endringer Pinto:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Peter Bjerring:** Conceptualization, Writing – review & editing, Funding acquisition. **Flemming Andersen:** Conceptualization, Writing – review & editing, Funding acquisition. **Christian Janfelt:** Methodology, Formal analysis, Investigation, Writing – review & editing, Resources. **Merete Haedersdal:** Conceptualization, Writing – review & editing, Funding acquisition, Resources. **Catharina Margrethe Lerche:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Funding acquisition, Resources, Supervision.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2024.100547.

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