International Journal of Food Sciences and Nutrition

ISSN: 0963-7486 (Print) 1465-3478 (Online) Journal homepage: www.tandfonline.com/journals/iijf20

Microbially mediated phenolic catabolites exert differential genoprotective activities in normal and adenocarcinoma cell lines

Brian Óg Murphy, Cheryl Latimer, Sara Dobani, L. Kirsty Pourshahidi, Massimilano Fontana, Nigel. G. Ternan, Gordon McDougall, Ian Rowland, Gema Pereira-Caro, Kieran M. Tuohy, Daniele Del Rio, Tahani M. Almutairi, Alan Crozier, Nenad Naumovski & Chris I. R. Gill

To cite this article: Brian Óg Murphy, Cheryl Latimer, Sara Dobani, L. Kirsty Pourshahidi, Massimilano Fontana, Nigel. G. Ternan, Gordon McDougall, Ian Rowland, Gema Pereira-Caro, Kieran M. Tuohy, Daniele Del Rio, Tahani M. Almutairi, Alan Crozier, Nenad Naumovski & Chris I. R. Gill (2024) Microbially mediated phenolic catabolites exert differential genoprotective activities in normal and adenocarcinoma cell lines , International Journal of Food Sciences and Nutrition, 75:7, 673-686, DOI: 10.1080/09637486.2024.2397055

To link to this article: https://doi.org/10.1080/09637486.2024.2397055

INTERNATIONAL JOURNAL OF FOOD SCIENCES AND NUTRITION
2024, VOL. 75, NO. 7, 673–686
https://doi.org/10.1080/09637486.2024.2397055

Taylor & Francis
Taylor & Francis Group

IN VITRO AND ANIMAL STUDIES

Microbially mediated phenolic catabolites exert differential genoprotective activities in normal and adenocarcinoma cell lines

Brian Óg Murphy[a], Cheryl Latimer[a], Sara Dobani[a], L. Kirsty Pourshahidi[a], Massimilano Fontana[a], Nigel. G. Ternan[a], Gordon McDougall[b], Ian Rowland[c], Gema Pereira-Caro[d], Kieran M. Tuohy[e], Daniele Del Rio[f], Tahani M. Almutairi[g], Alan Crozier[g,h], Nenad Naumovski[i] and Chris I. R. Gill[a]

[a]Nutrition Innovation Centre for Food and Health (NICHE), Ulster University, Coleraine, UK; [b]Environmental and Biochemical Sciences Department, The James Hutton Institute, Dundee, UK; [c]Department of Food and Nutritional Sciences, University of Reading, Reading, UK; [d]Department of Food Science and Health, IFAPA-Alameda Del Obispo, Córdoba, Spain; [e]School of Food Science and Nutrition, University of Leeds, Leeds, UK; [f]Human Nutrition Unit, Department of Food and Drug, University of Parma, Parma, Italy; [g]Department of Chemistry, King Saud University Riyadh, Saudi Arabia; [h]School of Medicine, Dentistry and Nursing, University of Glasgow, Glasgow, UK; [i]School of Rehabilitation and Exercise Sciences, Faculty of Health, University of Canberra, Canberra, Australia

ABSTRACT

Age-associated decline of nuclear factor erythroid 2-related factor 2 (Nrf2) activity and DNA repair efficiency leads to the accumulation of DNA damage and increased risk of cancer. Understanding the mechanisms behind increased levels of damaged DNA is crucial for developing interventions to mitigate age-related cancer risk. Associated with various health benefits, (poly)phenols and their microbially mediated phenolic catabolites represent a potential means to reduce DNA damage. Four colonic-microbiota-derived phenolic catabolites were investigated for their ability to reduce H_2O_2-induced oxidative DNA damage and modulate the Nrf2-Antixoidant Response Element (ARE) pathway, in normal (CCD 841 CoN) and adenocarcinoma (HT29) colonocyte cell lines. Each catabolite demonstrated significant ($p < .001$) genoprotective activity and modulation of key genes in the Nrf2-ARE pathway. Overall, the colon-derived phenolic metabolites, when assessed at physiologically relevant concentrations, reduced DNA damage in both normal and adenocarcinoma colonic cells in response to oxidative challenge, mediated in part *via* upregulation of the Nrf2-ARE pathway.

ARTICLE HISTORY
Received 18 March 2024
Revised 15 August 2024
Accepted 19 August 2024

KEYWORDS
Benzoic acid; colonic cells; COMET assay; 4-hydroxybenzoic acid; 3-(3′-hydroxyphenyl) propanoic acid; 3-(phenyl) propanoic acid

Introduction

The association between ageing and tumorigenesis is well-established, with life-time accumulation of DNA damage recognised as a significant contributing factor (Soria-Valles et al. 2017; Patel et al. 2020). As individuals age, the progressive accumulation of DNA damage within tissues becomes increasingly evident, potentially triggering either DNA repair, apoptosis or cell senescence, the latter a contributing factor to the decline in tissue function observed with age (Niedernhofer et al. 2018; Schumacher et al. 2021). In addition, the efficiency of DNA repair mechanisms declines over time, leading to the persistence of DNA damage within the genome as age advances (Gupta and Heinen 2019; Rashid et al. 2019; Schumacher et al. 2021). This accumulation of DNA damage is believed to directly contribute to the increase in cancer prevalence among older individuals (Patel et al. 2020). Diet and inflammation likely play critical roles in the accumulation of genomic alterations (Gómez de Cedrón et al. 2023). Poor dietary habits, characterised by high intake of processed foods, sugars and unhealthy fats, are linked to increased chronic inflammation (Falzone et al. 2022; Rudrapal et al. 2022). These diets promote the production of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) and activate Toll-like receptors (TLRs) on immune cells, stimulating inflammatory pathways and generating reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Falzone et al. 2022; Rudrapal et al. 2022). ROS are produced through various biochemical pathways, including mitochondrial respiration, NADPH oxidase activity and inflammatory cell activation (Krawczyk et al. 2023). Such reactive molecules can induce oxidative modifications in DNA, proteins and lipids, leading to mutations, impaired cellular

CONTACT Chris I. R. Gill c.gill@ulster.ac.uk Nutrition Innovation Centre for Food and Health (NICHE), Ulster University, Coleraine, UK
Supplemental data for this article can be accessed online at https://doi.org/10.1080/09637486.2024.2397055.

functions and activation of oncogenic pathways, which contribute to the progression of diseases including cancer (Krawczyk et al. 2023).

Conversely, diets rich in fruits, vegetables and whole grains, contain greater quantities of (poly)phenols, biologically active compounds such as quercetin, epigallocatechin gallate and resveratrol, which exhibit pleiotropic effects including anti-inflammatory, anti-microbial and anti-oxidant properties (Falzone et al. 2022; Rudrapal et al. 2022). Such compounds scavenge ROS, inhibit NF-κB activation and enhance DNA repair mechanisms, thereby reducing oxidative stress, DNA damage and inflammation, processes common to the pathogenesis of many chronic disease states (Gupta and Heinen 2019; Falzone et al. 2022; Rudrapal et al. 2022). Expanding our understanding of the mechanisms involved in age-related DNA damage accumulation could aid in the development of interventions to mitigate age-related cancer risk and improve overall health in the ageing population.

Polyphenols are a diverse group of bioactive aromatic phytochemicals with up to 10,000 different known structures, which play a role in reducing DNA damage (Durazzo et al. 2019; Di Lorenzo et al. 2021). Flavonoids, the most dietarily abundant (poly)phenols (Ahn-Jarvis et al. 2019; Khan et al. 2021; Rufino et al. 2021) are common to foods such as berries, coffee and cocoa (Del Rio et al. 2013), contributing to the estimated average European intake of 580–1780 mg of (poly)phenols per day (Zamora-Ros et al. 2016). Over the last decades, greatly improved analytical techniques have facilitated significant improvements in our understanding of both the metabolism and bioactive potential of these compounds (Gavrilas et al. 2019; Di Lorenzo et al. 2021; Dobani et al. 2021; Song et al. 2021).

Upon consumption, (poly)phenols undergo numerous structural changes during their transit through the gastrointestinal tract (GIT), including the production of bioactive, microbiota-derived phenolic catabolites (Carregosa et al. 2022), some of which have been reported to exert pleiotropic effects that likely contribute to reducing the risk for chronic diseases (Del Rio et al. 2013; Mena et al. 2019; Luca et al. 2020; Diotallevi et al. 2021; Rodríguez-Daza et al. 2021; Wang et al. 2022). However, the specific sites and mechanisms of action remain elusive, with multiple pathways postulated and currently under investigation. For example, the ability of (poly)phenolic-rich foods to affect aspects of the colorectal carcinogenesis pathway in gut epithelial cells has been studied *in vitro* (Brown et al. 2014; McDougall et al. 2017; Wang et al. 2022). The microbiota-derived catabolities of dietary (poly)phenols have been reported to modulate the response of pathways involved in the cell cycle and apoptosis, by inducing variation in expression of genes such as nuclear factor erythroid 2-related factor 2 (Nrf2), phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1) or BH3-interacting domain death agonist (BID) (Jin et al. 2010; Lu et al. 2018; Zakłos-Szyda et al. 2019; Song et al. 2021). Of particular interest is Nrf2, a redox-sensitive transcription factor master regulator of oxidoreductive and immune homeostasis. Under normal conditions cytosolic Nrf2 is associated with the inhibitory protein Kelch-like ECH-associated protein 1 (Keap1), which acts as a sensor of endogenous and exogenous prooxidants (Saha et al. 2020). Under a mild increase in oxidative stress, specific cysteine residues in Keap1 allow newly synthesised Nrf2 to escape Keap1-mediated ubiquitination and to activate the transcription of Nrf2-target genes. The protein then dimerises with small Maf proteins and binds to the antioxidant response element (ARE) in the promoter region of these genes, activating their transcription and eliciting antioxidant defence and detoxification mechanisms (Gavrilas et al. 2019). Consequently, Nrf2 represents an important potential therapeutic target in human carcinogenesis (Telkoparan-Akillilar et al. 2021) as it is activated by a range of diet derived (poly)phenolics (Islam et al. 2023). Moreover, ageing is associated with a decline in Nrf2 activity and signalling, resulting in a compromised cellular response to DNA damage (Schmidlin et al. 2019). A comprehensive review by Zhou et al. (2019) highlights the importance of (poly)phenols as activators of Keap1-Nrf2 signalling pathway, with many (poly)phenolic-rich berry fractions and to a lesser degree, their colonic catabolites reported to reduce DNA damage in various cell models (Prasad and Katiyar 2015; De Santiago et al. 2019; Majidinia et al. 2019; Maleki Dana et al. 2022).

Dobani et al. (2021) recently reported an ileostomate-based *ex vivo* model in which benzoic acid (BA), 4-hydroxybenzoic acid (4HBA), 3-(3′-hydroxyphenyl)propanoic acid (3'HPPA) and 3-(phenyl)propanoic acid (3PPA) were commonly produced as colonic catabolites following ingestion of raspberries, although they are not uniquely derived from that dietary source. Consequently, we investigated the differential effects of these hydroxylated and non-hydroxylated catabolites in two contrasting colonic cell lines with greater and lesser degrees of transformation (HT29 and CCD 841 CoN cells), with respect to DNA damage and ability to modulate key genes associated with the cytoprotective Nrf2-ARE pathway.

Material and methods

Chemicals and reagents

Foetal Bovine Serum (FBS), Minimum Essential Media (MEM), Dulbecco's Modified Eagle's Medium (DMEM) and phosphate-buffered saline (PBS) were purchased from Gibco Life Technologies Ltd (Paisley, Scotland, UK). All remaining chemicals including the phenolic catabolites 4HBA, BA, 3'HPPA and 3PPA were acquired from Sigma-Aldrich (Poole, Dorset, UK) unless otherwise stated.

Cell culture

Both normal epithelial (CCD 841 CoN) and adenocarcinoma (HT29) colonic cells used for this investigation were acquired from the American Type Culture Collection (ATCC) and European Collection of Cell Cultures (ECACC), respectively. CCD 841 CoN cells were cultured in MEM supplemented with 10% FBS, 100 U/L penicillin/streptomycin, 1% sodium pyruvate, 1% non-essential amino acids. The HT29 cells were cultured in DMEM supplemented with 10% FBS and 100 U/L penicillin/streptomycin. Both cell lines were grown as monolayers in tissue culture flasks as previously reported (Brown et al. 2014; De Santiago et al. 2019).

Cell treatments

Both cell lines were incubated without treatment (control) and individually with 3'HPPA, 3PPA, 4HBA or BA at 10, 50 and 100 µM, prepared from 5 mM stock solutions as previously detailed (Dobani et al. 2021).

Cytotoxicity assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was used to determine cell viability. Following a previously established protocol (Brown et al. 2014), cells (1.5 × 104 cells/well) were seeded in a 96 multi-well plate and incubated for 48 h (37 °C, 5% CO_2) before replacement of media with 100 µL of each treatment and incubation (37 °C, 5% CO_2). After 24 h, cells were washed with PBS, treatments were replaced with an equal volume of media and 15 µL of MTT (5 mg/mL) was added to each well. After a 4 h incubation (37 °C, 5% CO_2) and addition of the solubilising solution, prepared as previously reported (Saha et al. 2020; Carregosa et al. 2022), absorbance at 570 nm was measured with a microplate-reader (VERSAmax). Experiments were carried out in triplicate and mean results expressed as a percentage (%) of cell viability normalised to untreated control. Deoxycholic acid (250 mM) was used as negative control and threshold cell viability level was set at 50%.

Genotoxicity assay

The COMET assay was used to determine each phenolic's efficacy in reducing DNA damage in CCD 841 CoN and HT29 cell lines. As outlined by De Santiago et al. (2019), both cell lines were incubated with each phenolic compound individually for 24 h at 37 °C in 5% CO_2 before undergoing oxidative challenge with either 75 µM hydrogen peroxide (H_2O_2) for HT29 cells or 25 µM hydrogen peroxide (H_2O_2) for CCD841 CoN cells to elicit an equivalent amount of damage (~50% Tail DNA) in the untreated controls. Images were analysed using a Nikon Eclipse 600 epifluorescence micro-scope at 400× magnification, with Komet 5.0 software used to record the tail DNA (%). The genoprotective potential was assessed by comparing the effects of the phenolics against the positive (H_2O_2) control and genotoxic activity by comparing against the negative (PBS) experimental control. Experiments were carried out in triplicate and mean results expressed as a percentage Tail DNA.

Real-time PCR (qPCR)

Following the previously established method (Dobani et al. 2021), primers (Table 1) were designed for three

Table 1. Oligonucleotide primers used for PCR amplification and qPCR to assess the change in expression after treatment.

Gene	Full name	Forward primer	Sequence (5′→3′)	Reverse primer	Sequence (3′→5′)	Product size (bp)
Nrf2	Nuclear factor (erythroid-derived 2)-like 2	Nrf2-F	AAACCATGGGACTGCCCAAC	Nrf2-R	GCACTAGGAAGACTGGGCTCTC	190
HO-1	Haem oxygenase 1	HO-1-F	ATGACACCAAGGACCAGAGC	HO-1-R	GTGTAAAGGACCCATGGCAGA	153
NQO1	NAD(P)H dehydrogenase, quinone 1	NQO1-F	AGGACCCTTCCGGAAGTAAGA	NQO1-R	AGGCTGCTTGGGAGCAAAATA	279
HPRT	hypoxanthine phosphoribosyltransferase	HPRT-F	AGCTTGCGACCTTGACCAT	HPRT-R	GACCAGTCAACAGGGGACAT	166
β-Actin	Actin, beta	β-Actin-F	GGACTTCGAGCAAGAGATGG	β-Actin-R	AGCACTGTGTTGGCGTACAG	234

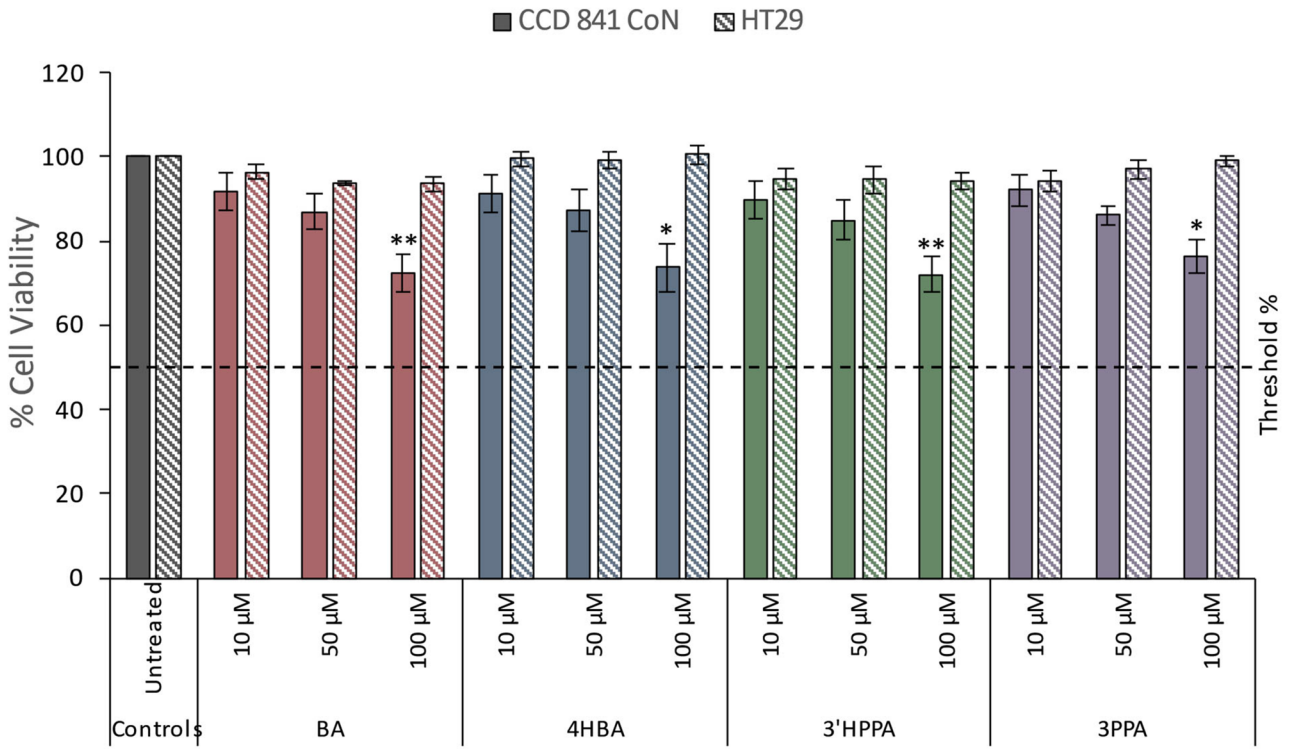

Figure 1. Cytotoxic effect of different concentrations of phenolics on CCD 841 CoN and HT29 cells. Data are presented as mean of three independent experiments ± SEM. Significance is compared to untreated control using one-way ANOVA and Dunnett's multiple comparison test, *$p < .05$, **$p < .01$. BA: benzoic acid; 4HBA: 4-hydroxybenzoic acid; 3'HPPA: 3-(3'-hydroxyphenyl)propanoic acid; 3PPA: 3-(phenyl)propanoic acid.

genes of interest involved in the Nrf2-ARE pathway; Nrf2, Haem oxygenase-1 (HO-1) and NAD(P)H:quinone oxidoreductase 1 (NQO1), utilising the online software tools OligoPerfect (Thermo Fisher, UK) and NCBI Primer-BLAST (Ye et al. 2012). The following cycling condition were applied; 95 °C for 10 min, followed by 50 cycles of 95 °C for 10 s, 57 °C for 10 s and 72 °C for 10 s. Expression for each target gene was normalised against two reference genes (i.e. hypoxanthine phosphoribosyltransferase (HPRT) and β-actin) and analyses were performed in triplicate and data was expressed as the logarithmic of fold change.

Statistical analysis

The results of three independent experiments were used for statistical analysis and presented as means ± standard error of the mean (SEM) for each type of dataset. Normality of datasets was assessed with the D'Agostino and Pearson omnibus test. One-way ANOVA, with Dunnett's multiple comparison post-hoc test, was used to assessed significant differences between means ($n = 3$) compared to control (untreated cells) and a Tukey's multiple comparison post-hoc test to compare concentration effects within compound for results of cytotoxicity, genoprotective activity and gene expression. Additionally, Tukey's multiple comparisons were also used to assess significance between the different compounds at the same concentrations for both genoprotective activity and gene expression. An unpaired t-test was used to determine significance between cell lines for genotoxicity and gene expression. Significance was set at $p < .05$. Analyses were performed using Prism 9 (Version 9.5.0 for Windows).

Results

Cell viability

None of the phenolic compounds (0–100 µM) tested significantly influenced the viability of HT29 cells (Figure 1), whereas cell viability for CCD 841 CoN cells was decreased to ~75% at the highest concentration tested of each phenolic. Consistent with Dobani et al. (2021), the dose range (0–100 µM) was considered to be sub-cytotoxic for the purposes of the remaining experiments.

DNA damage reduction

All four phenolics, 3'HPPA, 3PPA, 4HBA and BA, reduced oxidative-induced DNA damage in HT29 and

CCD 841 CoN cells. In the HT29 cell line, the benzoic acids showed different patterns of effect (Figure 2(A,B)) with BA exerting a dose-dependent response with maximal effect at 100 µM, where BA reduced DNA damage to 26.9% tail DNA (F (4, 10) = 25.7, $p < .001$), followed by 50 µM BA (34.8%, F (4, 10) = 25.7, $p < .001$) and 10 µM BA (43.4%, F (4, 10) = 25.7, $p < .05$) (Figure 2(A)). In contrast, 4HBA exhibited more genoprotection at the lowest concentration (10 µM) where it produced a threshold effect, resulting in reductions in DNA damage to 29.9% (F (4, 10) = 19.3, $p < .001$) and reduced activity seen at the higher concentrations (32.2% tail DNA at 50 µM and 31.7% tail DNA at 100 µM) (Figure 2(B)). For the phenylpropanoic acids (3PPA and 3'HPPA; Figure 2(C,D)), the most potent effect in the HT29 cells was observed at 50 µM with DNA damage reduced to 32.2% (F (4, 10) = 72.2, $p < .001$) and 34.4% (F (4, 10) = 43.8, $p < .001$), respectively, while higher concentrations were less genoprotective. However, it was notable that 3'HPPA caused a significant reduction in DNA damage at 10 µM (43.3%, F (4, 10) = 43.8, $p < .01$) and was significantly (F (3, 8) = 10.5, $p < .05$) different in comparison to 3PPA which had no observable effect (48.4%) (Figure 2(D)).

In the CCD 841 CoN cell line, BA exhibited a dose-dependent response with maximal effect at 100 µM, reducing DNA damage to 24.6% (F (4, 10) = 50.7, $p < .001$), followed by 50 µM BA (35.0%, F (4, 10) = 50.7, $p < .001$) and 10 µM BA (40.3%, F (4, 10) = 50.7, $p < .05$) (Figure 2(A)). 4HBA was most potent at the highest 100 µM concentration (25.1%, F (4, 10) = 32.8, $p < .001$) but interestingly was similarly effective at the lowest dose 10 µM (31.8%, F (4, 10) = 32.8, $p < .001$) (Figure 2(B)). The hydroxylated form is therefore more potent than the non-hydroxylated BA. For the phenylpropanoic acids, 3PPA and 3'HPPA (Figure 2(C,D), respectively), the most potent effect was observed at 100 µM (reductions to 24.7% and 22.5%, respectively; F (4, 10) = 45.1 and 43.1, respectively, $p < .001$), however at the lowest concentration (10 µM) only 3'HPPA exerted a significant reduction

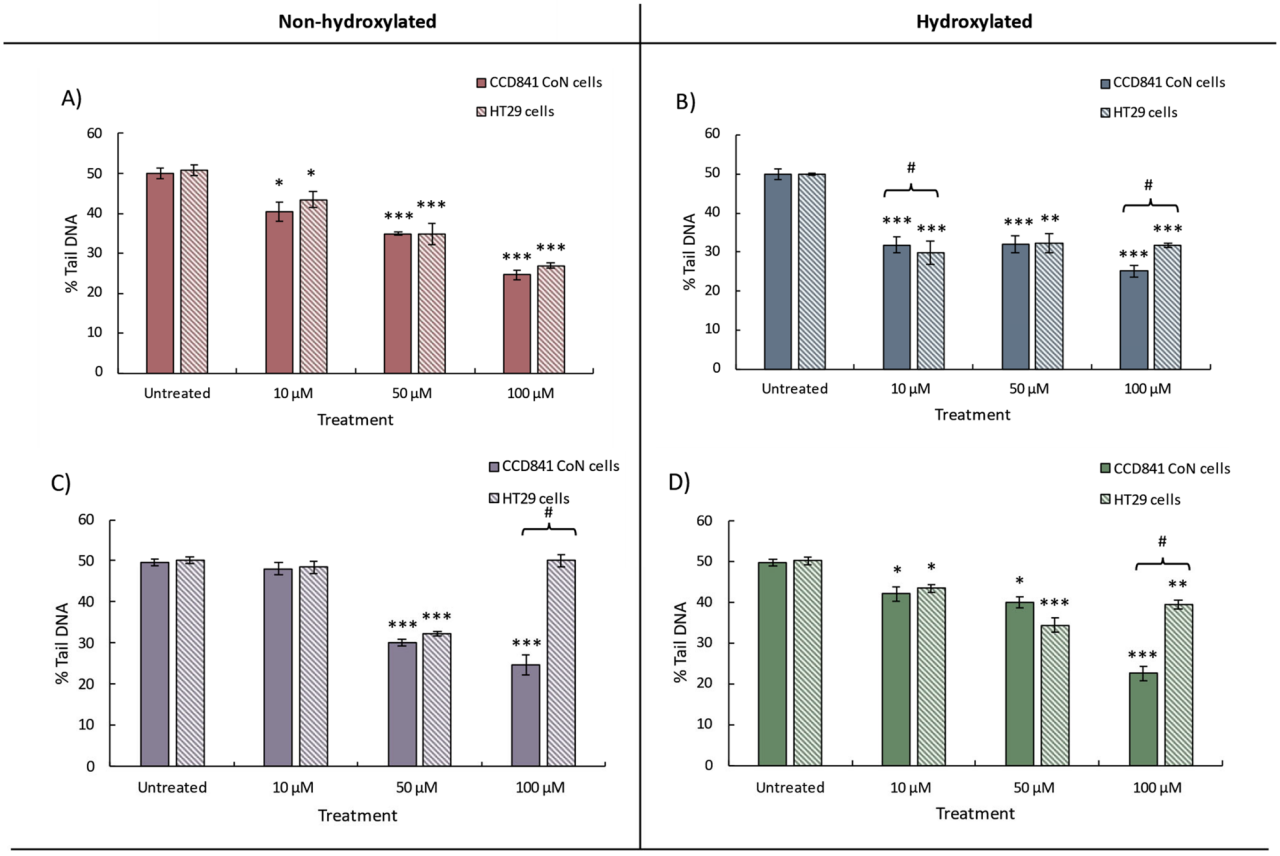

Figure 2. DNA damage reducing capacity of four individual phenolics on CCD 841 CoN and HT29 cells in response to oxidative challenge. Data for (A) benzoic acid (BA), (B) 4-hydroxybenzoic acid (4HBA), (C) 3-(phenyl)propanoic acid (3PPA) and (D) 3-(3'-hydroxyphenyl)propanoic acid (3'HPPA), at 10, 50 or 100 µM after 24 h pre-incubation of cells at 37 °C and challenge with H_2O_2 (75 µM – HT29 or 25 µM – CDD 841 CoN) are presented. Data are presented as mean of three independent experiments ± SEM. Significance is compared to untreated control using one-way ANOVA and Dunnett's multiple comparison test, *$p < .05$, **$p < .01$, ***$p < .001$, significance compared between cell types using an independent t-test, #$p < .05$.

in DNA damage compared to control (42.0% vs 49.6%; F (4, 10) = 43.1, $p < .05$). This could indicate that 3'HPPA is the more potent compound, however, at 50 µM, 3PPA was significantly more effective in preventing DNA damage (F (3, 8) = 6.67, $p < .05$) than 3'HPPA.

The phenolic compounds under investigation did not exert significant genotoxic damage compared to the negative experimental control in either cell line (data not shown) but did significantly reduce DNA damage (% Tail DNA) in response to oxidative challenge. 4HBA was more potent than BA in both cell lines, causing significant reduction in DNA damage even at lower concentrations. The effect of hydroxylation on DNA damage reduction was less consistent for the phenylpropanoic acids as 3PPA did not significantly decrease in DNA damage at 10 µM in either cell line nor at the highest dose (100 µM) for HT29 in contrast to the pattern observed for 3'HPPA (Supplementary Table 1).

Modulation of gene expression within the Nrf2-pathway

Addition of the phenolic acids resulted in an increase in the measured expression of Nrf2 in both cell lines compared to the untreated control (Figure 3(A–D)). Changes in Nrf2 expression were generally similar between cell lines, however higher phenolic concentrations (50 and 100 µM) elicited significant increases (F (4, 10) = 259, $p < .05$) in Nrf2 expression above the controls. A dose effect was observed in HT29 cells treated with BA (maximum 2.37-fold increase, 100 µM, $p < .001$; Figure 3(A)), however an inverse correlation between dose and expression was seen for 4HBA with no significant change from the control at 100 µM (Figure 3(B)). An exception to these general observations was in HT29 adenocarcinoma cells, where the addition of 3PPA resulted in highly significant (F (4, 10) = 521 $p < .001$) decreases in Nrf2 expression at both 10 µM and 100 µM, but with a highly significant ($p < .001$) increase at 50 µM (Figure 3(C)).

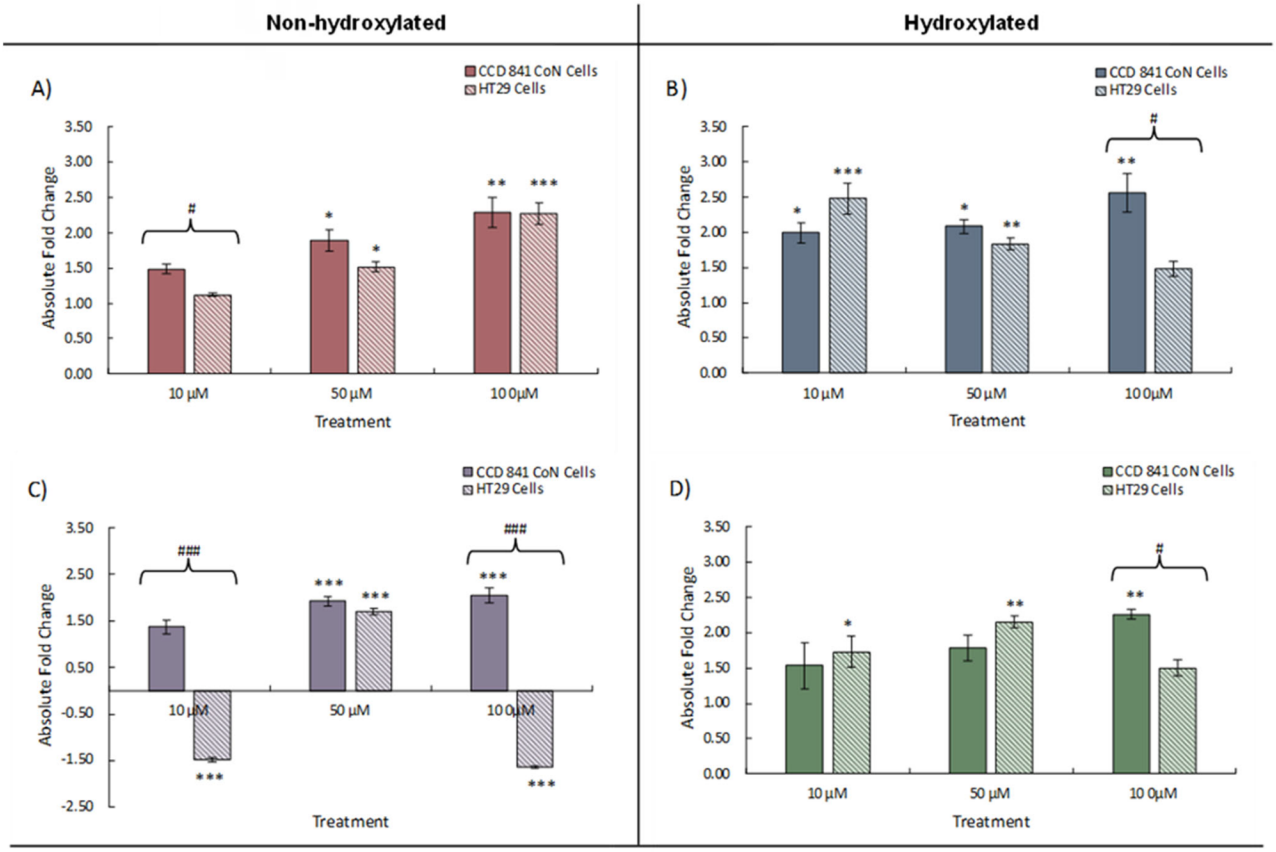

Figure 3. Change in Nrf2 expression in CCD 841 CoN and HT29 cells treated with either 10, 50 or 100 µM of individual phenolic compounds. Data for (A) benzoic acid (BA), (B) 4-hydroxybenzoic acid (4HBA), (C) 3-(phenyl)propanoic acid (3PPA) and (D) 3-(3'-hydroxyphenyl)propanoic acid (3'HPPA) are presented as mean of three independent experiments ± SEM. Significance is compared to untreated control using one-way ANOVA and Dunnett's multiple comparison test, *$p < .05$, **$p < .01$, ***$p < .001$, significance compared between cell types using an independent t-test, #$p < .05$, ###$p < .001$.

By comparison, the CCD 841 CoN normal cell line exhibited increases in Nrf2 expression under the same treatments and the observed difference suggests less well-regulated and/or responsive gene expression in the carcinoma cell line – in the presence of 3PPA – than normal CCD 841 CoN cells. It is known that Nrf2 binds to the ARE element upstream of target genes and increases expression of antioxidant defence/detoxification functions within the cell (Saha et al. 2020) and we therefore measured expression of NQO1 and HO-1 in the cell lines under investigation.

NQO1 scavenges ROS and is implicated in reducing DNA damage (Rashid et al. 2021) and, in our investigation, NAD(P)H:quinone oxidoreductase 1 (NQO1) expression, similar to Nrf2 expression, was increased by the addition, individually, of all four phenolic acids to both cell lines (Figure 4(A–D)). The less transformed CCD 841 CoN cells exhibited increased NQO1 expression with increasing phenolic concentration with the most pronounced dose effect observed with 3PPA (Figure 4(C)). Similar to the Nrf2 data, decreases in NQO1 expression were measured in HT29 cells in the presence of 3PPA, possibly as a consequence of the apparently dysregulated Nrf2 expression also observed in these cells with this treatment. We also noted decreased expression (1.5-fold) of NQO1 in HT29 cells exposed to 100 µM 4HBA, contrasting with a 1.5-fold increase in expression in CCD 841 CoN cells exposed to the same treatment (Figure 4(B)). Statistically significant differences in NQO1 expression between CCD841 and HT29 cells again mapped – broadly – to the same phenolic treatments where such differences were observed in Nrf2 expression, and the data suggests that the greatest differences in gene expression between cells occurs at both the lowest and highest phenolic concentrations.

Haem oxygenase (HO-1) is an inducible enzyme that exerts cytoprotective effects, as the products of its catabolism of haem possess antioxidative, anti-inflammatory and antiapoptotic activities (Zhang et al. 2021). For this gene, we again observed a similar response to treatment with increases in expression arising from treatment of both cell lines (Figure 5(A–D)). Divergence in HO-1 expression between CCD

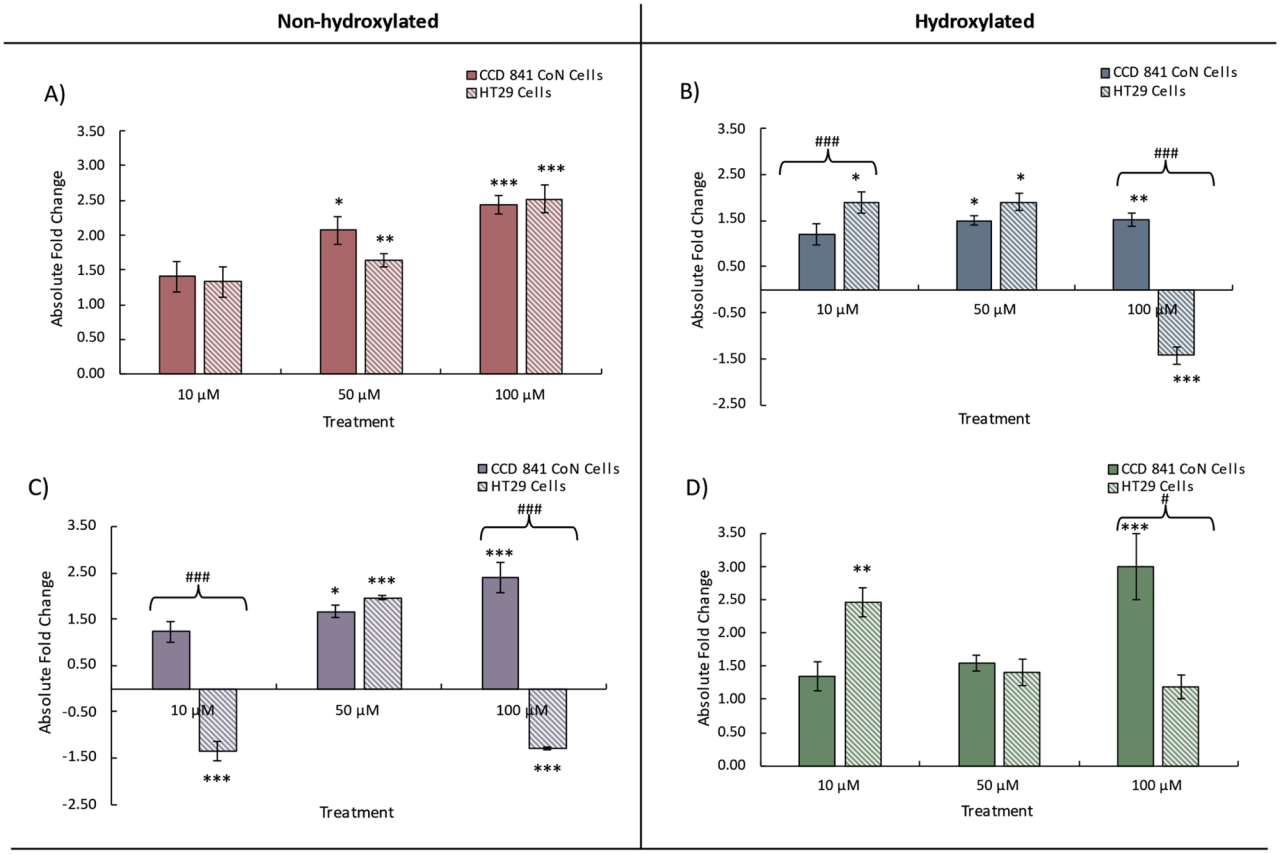

Figure 4. Change in NQO1 expression in CCD 841 CoN and HT29 cells treated with either 10, 50 or 100 µM individual phenolics; (A) benzoic acid (BA), (B) 4-hydroxybenzoic acid (4HBA), (C) 3-(phenyl)propanoic acid (3PPA) and (D) 3-(3′-hydroxyphenyl)propanoic acid (3′HPPA). Data presented are as mean of three independent experiments ± SEM. Significance is compared to untreated control using one-way ANOVA and Dunnett's multiple comparison test, *$p < .05$, **$p < .01$, ***$p < .001$, significance compared between cell types using an independent t-test, #$p < .05$, ###$p < .001$.

841 CoN (increase) and HT29 cells (decrease) with 10 µM and 100 µM 3PPA was observed (Figure 5(C)), consistent with our observation for Nrf2 and NQO1 (Figures 3(A–D) and 4(A–D), respectively). We also noted a relatively greater maximum increase in HO-1 expression with BA (Figure 5(A)) and 4HBA (Figure 5(B)) and for 3'HPPA (Figure 5(D)), with the maximal increases – with the exception of 3PPA (50 µM; Figure 5(C)) – observed following treatment with 100 µM of a given phenolic. With the exception of the divergence in expression between cell lines, expressional changes following treatment with 3PPA were more modest (~1.5-fold) (Figure 5(C)) an observation made also for both Nrf2 (Figure 3(A–D)) and NQO1 (Figure 4(A–D)) expression. 3'HPPA at the lowest phenolic concentration (10 µM) consistently exhibited no significant changes to gene expression for the normal cell line verses HT29 cells. Moreover, 3PPA and 4HBA exerted decrease in genes expression in HT29 cells whereas the normal cells were unaffected to responded with an increase in gene expression. Overall only limited differences were observed between the hydroxylated and non-hydroxylated phenolics' ability to modulate the expression of each of the genes were evident and no discernible pattern was evident (Supplementary Table 1).

Taken together, our data indicate that each of the phenolic compounds tested reduce DNA damage in colonic cells in response to an oxidative stress challenge. This effect appears mediated, in part, by altered gene expression of Nrf2, NQO1 and HO-1 genes. Some concentration and or structure dependent effects were apparent in the response of the more and less transformed cell lines to the phenolic acids tested. For example, phenylpropanoic acid treatments (PPA and 3'HPPA) caused noticeably different patterns of response in both cell lines (except 50 µM), while benzoic acids (BA and 4HBA) elicited similar levels of gene expression except at the highest concentration of 4HBA (Figure 3(B)).

Discussion

Reducing damage to DNA is a critical cellular defence mechanism that safeguards against mutations and

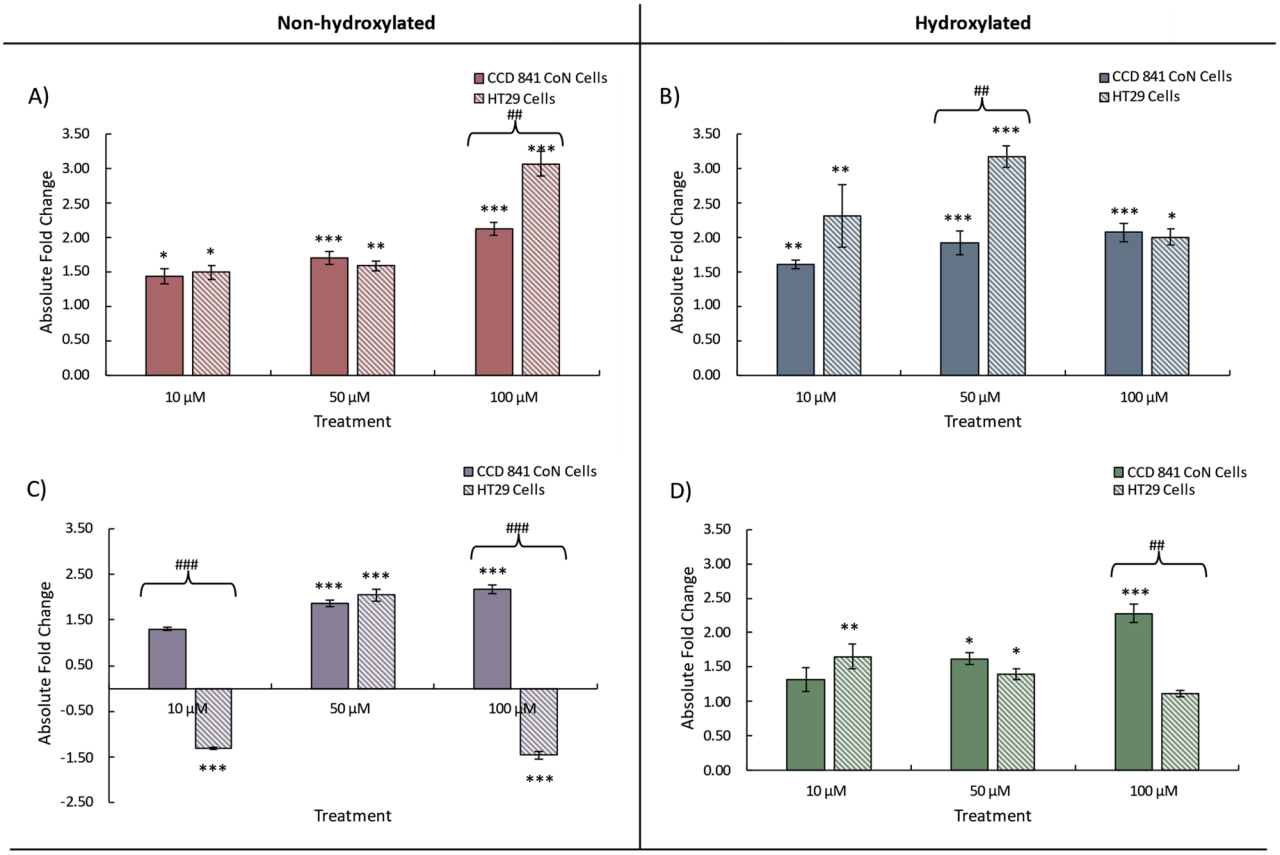

Figure 5. Change in HO-1 expression in CCD 841 CoN and HT29 cells treated with either 10, 50 or 100 µM individual phenolics; (A) benzoic acid (BA), (B) 4-hydroxybenzoic acid (4HBA), (C) 3-(phenyl)propanoic acid (3PPA) and (D) 3-(3'-hydroxyphenyl)propanoic acid (3'HPPA). Data presented are as mean of three independent experiments ± SEM. Significance is compared to untreated control using one-way ANOVA and Dunnett's multiple comparison test, *$p < .05$, **$p < .01$, ***$p < .001$, significance compared between cell types using an independent t-test, ##$p < .01$, ###$p < .001$.

maintains normal cellular functions (Izquierdo-Vega et al. 2017). Extensive research has established that (poly)phenols could positively contribute in maintaining a healthy status by influencing this mechanism along with exerting anti-proliferative, anti-inflammatory and anti-oxidative effects (Moura et al. 2013; Coșarcă et al. 2019; Patra et al. 2021; Pereira-Caro et al. 2023). As a result, there has been increased interest in determining the mechanism of actions of these plant-derived compounds and more recently their catabolites, at both the cellular and systemic level.

Due to their extensive biotransformation, especially in the distal gut, which alters (poly)phenol bioavailability and biological activity, it is important to test dietarily relevant phytochemicals at physiologically relevant doses and in the chemical forms encountered by different human tissues (Del Rio et al. 2013; Mena et al. 2019). Phenolic acids are present both in the food sources and as catabolites derived from their in planta parent (poly)phenols (Brown et al. 2014; McDougall et al. 2017; Mena et al. 2019; Luca et al. 2020; Diotallevi et al. 2021; Rodríguez-Daza et al. 2021; Wang et al. 2022) formed during catabolism by the intra-luminal microbiota (Carregosa et al. 2022), as well as from the putative metabolism of other compounds such as phenylalanine and catecholamines (Pereira-Caro et al. 2023). Compositional analyses of *in vivo* digestion or *in vitro* faecal fermentations can determine the physiological relevance of these compounds to the colon (Brown et al. 2012; Dobani et al. 2021). For example, Dobani et al. (2021) highlighted a clear differentiation between pre- and post-fermentation ileal fluid samples, with eight raspberry-derived phenolic acid catabolites identified as key discriminators of the fermentation process, four of which were assessed for their genoprotective potential and the ability to modulate genes in the cyto-protective Nrf2 mediated ARE pathway in normal colonocytes (CCD 841 CoN).

In the current study, the work by Dobani and colleagues on the genoprotective effects of the simple phenolics (3PPA, 3'HPPA, BA, 4'HBA) on the CCD 841 CoN cells (Dobani et al. 2021) has been expanded using the classic and more transformed colonocyte cell line HT29. This cell line exhibits rapid growth, irregular morphology and carries genetic alterations associated with colorectal adenocarcinoma (Pearce et al. 2018). In contrast, CCD 841 CoN cells are derived from normal cells, display slower growth, epithelial-like morphology and retain the genetic and phenotypic characteristics of healthy colonic epithelium (Zoetemelk et al. 2019). Heterogeneities in both physiological and biochemical response have been report-ed between these cell lines when testing the same compound under similar conditions (Pan et al. 2021) and, in the current work, the individual phenolic acid treatments exerted greater genoprotective capacity in the CCD 841 CoN cell line, particularly at the highest concentrations of 4HBA, 3PPA and 3'HPPA. By contrast, a recent *ex vivo* study investigating strawberry enriched ileal fluid fermentates in the same cell lines, determined that a complex mixture of (poly)phenolic compounds was less effective in reducing DNA damage in CCD 841 CoN cells than HT29 cells in response to oxidative challenge (Diotallevi et al. 2021). An investigation that assessed a colonically relevant raspberry-derived triterpenoid extract elucidated that the extract exerted greater anti-genotoxic capacity in the CCD 841 CoN cells than HT29 cells, reducing DNA damage by ~55% and ~45%, respectively (McDougall et al. 2017). Overall, the data from the current investigation are generally consistent with previous work in our laboratory (Brown et al. 2012), in which HT29 cells treated with 5 µg/mL of 3'HPPA (30 µM) or 4HBA (~36 µM) exhibited a reduction in DNA damage of 30% in response to oxidative challenge (75 µM H_2O_2), similar to the magnitude of effect reported here. It has been reported that açai and blueberry extracts (2.5 mg/mL), which are also sources of simple phenolic compounds like 4HBA, in addition to other polyphenolics such as pelargonidin-3-O-glucoside, reduced DNA damage in HT29 cells by ~60% ($p < .001$) compared to the untreated control (Randah et al. 2018). What are arguably the physiologically more relevant effects were revealed when the *in vitro* digested and fermented acai extract was tested, where it significantly reduced DNA damage by ~31.5% (Alqurashi et al. 2017).

The gut microbiota plays a pivotal role in the modulation of (poly)phenols and their colonic catabolites (Brown et al. 2014; McDougall et al. 2017; Luca et al. 2020). The phenolic acids used in this study are present during colonic transit at differing concentrations and in differing forms, differentiated through the presence/absence of a hydroxyl group. Hydroxylated phenolics have been reported to have higher bioavailability and bioactivity (Lee et al. 2023) and this may be corroborated by comparison of DNA damage between the hydroxylated and non-hydroxylated phenolics in each cell line. Treatment with both 4HBA and 3'HPPA resulted in greater reductions in DNA damage, particularly at lower concentrations, than either BA or 3PPA, in addition to their higher level of gene modulation, particularly in the adeno-carcinoma cells. This observation may be due to greater antioxidant activity and indeed a recent study (Chiang et al.

2016) reported that the microbially derived 3′-hydroxylated soy isoflavone derivatives, 3′-hydroxydaidzin and 3′-hydroxygenistin, exhibited higher free radical scavenging activity than their non-hydroxylated counterparts. In addition, Cao et al. (1997) reported stronger antioxidant activity in flavonoids with hydroxyl groups at both the 3′ and 4′ positions of the B-ring in comparison to their dehydroxylated counterparts. Our observations, therefore, may be attributable to the increased antioxidant activity seen with the hydroxylated phenolic acids, as hydroxyl groups are reported as having strong radical-scavenging and antioxidant effects (Umeno et al. 2016; Kostić et al. 2023). As both the hydroxylated and non-hydroxylated forms provide genoprotective effects *in vitro* at physiological levels, this suggests that they could provide synergistic protection following their colonic generation *via* the successive microbial degradation of intact flavonoids to the simple phenolic acids that accumulate and are excreted in the faeces.

The underlying mechanism behind the antigenotoxic effects of these compounds is not fully understood, however, accumulating evidence (Yoshimura et al. 2016; Mou et al. 2017; Bajpai et al. 2019; Silva et al. 2019; Darband et al. 2020; Moratilla-Rivera et al. 2023) suggests that the Nrf2-ARE pathway may play a role in protecting against DNA damage by activating down-stream targets such as NQO1 and HO-1, potent anti-inflammatory enzymes that alleviate oxidative stress in cells (Saha et al. 2020). The current study clearly highlights the role each phenolic acid may play in modulating the expression of Nrf2 and its target genes, NQO1 and HO-1 in both normal colonic epithelial cells (CCD 841 CoN) and carcinogenic colonic epithelial cells (HT29). The hydroxylation status of the phenolic compound tested appears to exert some differential effects on gene expression for HO1 and NQO1 but not for Nrf2. For Nrf2, no clear pattern of effect was evident, for example PPA and 3'HPPA caused noticeably different patterns (increase vs decrease) while benzoic acids elicited similar patterns and levels of gene expression in both cell lines. This is in broad agreement with the previous finding of McDougall et al. (2017) who demonstrated that a raspberry triterpenoid-rich fraction caused increased expression of Nrf2 in HT29 cells but, conversely, decreased it in CCD 841 CoN cells, while measured NQO1 (increase) and HO-1 (decrease) gene expression was similar in both cell lines. More recently, research in hepatocyte cells (HepG2) (Chen et al. 2019) demonstrated that a solvent-based raspberry extract (200 µg/mL) – albeit at physiologically questionable levels – increased Nrf2, NQO1 and HO-1 gene expression by 38.4% ($p < .01$), 18.7% ($p < .01$) and 9.4% relative to the control. (Poly)phenols, such as resveratrol, cyanidin, chlorogenic acid and luteolin also activate the Nrf2-ARE pathway and exert antigenotoxic effects (Teng et al. 2017; Kang et al. 2019). Indeed, luteolin (60 µM) reduced H_2O_2 induced ROS generation (at 24 h) by 50% in a dose-dependent manner, mediated in part by significant ($p < .05$) time dependent (12, 24 and 48 h) increases in Nrf2 expression (~15-, 50- and 145-fold increased mRNA levels, respectively) in HT29 cells (Kang et al. 2019).

Polyphenols also interact with intestinal microbiota modulating of composition, promoting the growth of bacteria such as *Lactobacillus*, *Akkermansia* and *Bifidobacterium* (Rodríguez-Daza et al. 2021), while suppressing pathogenic bacteria like *Escherichia coli* and *Bacteroides fragilis* (Zhao et al. 2023). Such alterations of the gut microbiota exert putative anti-cancer effects including direct tumour suppression and modulation of the immune system (Li et al. 2024; Zhao et al. 2023; Masheghati et al. 2024). For example, hydrocaffeic acid and urolithin A have been shown to exhibit anti-inflammatory and anti-cancer activities by upregulating tight junction proteins (e.g. ZO-1, occludin) through pathways like AhR and Nrf2, thereby protecting gut barrier integrity and inhibiting proinflammatory cytokines (Zhao and Jiang 2021). While a polyphenol-rich cranberry extract exerted a prebiotic effect increasing *Akkermansia muciniphila*, stimulating mucin production *via* MUC2 expression and promoting expression of tight junction proteins such as occludin and claudin, thereby improving barrier function and reducing chronic inflammation of the intestinal cells (Chelakkot et al. 2018; Yu et al. 2022; Li et al. 2024). More recently Lessard-Lord et al. (2024) demonstrated cranberry extract improving MUC2 and TJP1 although this was associate with increases bifidobacterium and the decrease of bacteroides rather than *Akkermansia*. Therefore, the current study builds upon our previous work, and that of others, albeit limited as it uses only a single cancer cell line (HT29) to contrast against the Con CD841 cell line, nonetheless, the results emphasise the role of (poly)phenols in inducing phase II detoxifying and antioxidant enzymes, regulating oxidative stress in both normal and adenocarcinoma cells. However, the effects vary depending on the compound, concentration and cell type. Future research should seek to further explore these findings, utilising multiple carcinogenic cells lines with long-term exposure to the individual phenolics. Moreover, further research is needed to evaluate the production of lactate dehydrogenase and glutathione to further elucidate the proposed protective effects.

Conclusions

This study provides evidence that microbially mediated phenolic catabolites possesses the ability to reduce DNA damage induced by oxidative challenge in two colonic epithelial models (CCD841 CoN and HT29 colonic cell lines). The protective effect is partially attributed to the upregulation of the Nrf2-ARE pathway and its downstream targets, NQO1 and HO-1. Despite this, the hydroxylation state of the phenolic acids appears to have limited impact on the bioactivity overall. Consequently, the presence of abundant amounts of low molecular weight phenolic catabolites from diets rich in (poly)phenols may exert protective effects against colonic epithelial damage *in vivo* by reducing DNA damage, a key early-stage event in colorectal carcinogenesis.

Authors contributions

C.I.R.G., G.McD., I.R. and A.C. were involved in study design. B.Ó.M. S.D., M.F. and C.L., were involved in experimental and data analysis. The manuscript was prepared by B.Ó.M., T.M.A, C.L., S.D., G.P.-C., K.M.T, N.G.T., L.K.P., D.D.R., C.L., I.R., G.McD., A.C., N.K. and C.I.R.G with contributions from all the authors.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

C.I.R.G. and A.C. received funding from the National Processed Raspberry Council who had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. G.McD. received funding from the Scottish Government's Rural and Environment Science and Analytical Services (RESAS) Division. G.McD. received funding from BachBerry (Project No. FP7-613793). G.P.-C. was supported by a postdoctoral research contract "Juan de la Cierva Incorporación" funded by the Spanish Ministry of Economy and Competitiveness (FJCI-2015-26433). C.I.R.G., A.C., G.P.-C. and T.M.A. were funded by the Distinguished Scientist Fellowship Program (DSFP) of King Saud University, Riyadh, Saudi Arabia.

References

Ahn-Jarvis JH, Parihar A, Doseff AI. 2019. Dietary flavonoids for immunoregulation and cancer: food design for targeting disease. Antioxidants. 8(7):202. doi: 10.3390/antiox8070202.

Alqurashi RM, Alarifi SN, Walton GE, Costabile AF, Rowland IR, Commane DM. 2017. In vitro approaches to assess the effects of açai (*Euterpe oleracea*) digestion on polyphenol availability and the subsequent impact on the faecal microbiota. Food Chem. 234:190–198. doi: 10.1016/j.foodchem.2017.04.164.

Bajpai VK, Alam MB, Ju MK, Kwon K-R, Huh YS, Han Y-K, Lee SH. 2019. Antioxidant mechanism of polyphenol-rich *Nymphaea nouchali* leaf extract protecting DNA damage and attenuating oxidative stress-induced cell death via Nrf2-mediated heme-oxygenase-1 induction coupled with ERK/p38 signaling pathway. Biomed Pharmacother. 103:1397–1407. doi: 10.1016/j.biopha.2018.04.186.

Brown EM, McDougall GJ, Stewart D, Pereira-Caro G, González-Barrio R, Allsopp P, Magee P, Crozier A, Rowland I, Gill CIR. 2012. Persistence of anticancer activity in berry extracts after simulated gastrointestinal digestion and colonic fermentation. PLoS One. 7(11):e49740. doi: 10.1371/journal.pone.0049740.

Brown EM, Nitecki S, Pereira-Caro G, McDougall GJ, Stewart D, Rowland IR, Crozier A, Gill CIR. 2014. Comparison of in vivo and in vitro digestion on polyphenol composition in lingonberries: potential impact on colonic health. Biofactors. 40(6):611–623. doi: 10.1002/biof.1173.

Cao G, Sofic E, Prior RL. 1997. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radic Biol Med. 22(5):749–760. doi: 10.1016/s0891-5849(96)00351-6.

Carregosa D, Pinto C, Ávila-Gálvez MÁ, Bastos P, Berry D, Santos CN. 2022. A look beyond dietary (poly)phenols: the low molecular weight phenolic metabolites and their concentrations in human circulation. Compr Rev Food Sci Food Saf. 21(5):3931–3962. doi: 10.1111/1541-4337.13006.

Chelakkot C, Choi Y, Kim DK, Park HT, Ghim J, Kwon Y, Jeon J, Kim MS, Jee YK, Gho YS, et al. 2018. *Akkermansia muciniphila*-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. Exp Mol Med. 50(2):e450–e450. doi: 10.1038/emm.2017.282.

Chen L, Li K, Liu Q, Quiles JL, Filosa R, Kamal MA, Wang F, Kai G, Zou X, Teng H, et al. 2019. Protective effects of raspberry on the oxidative damage in HepG2 cells through Keap1/Nrf2-dependent signalling pathway. Food Chem Toxicol. 133:110781. doi: 10.1016/j.fct.2019.110781.

Chiang CM, Wang D-S, Chang T-S. 2016. Improving free radical scavenging activity of soy isoflavone glycosides daidzin and genistin by 3′-hydroxylation using recombinant *Escherichia coli*. Molecules. 21(12):1723. doi: 10.3390/molecules21121723.

Coșarcă S, Moacă E, Tanase C, Muntean D, Pavel I, Dehelean C. 2019. Spruce and beech bark aqueous extracts: source of polyphenols, tannins and antioxidants correlated to in vitro antitumor potential on two different cell lines. Wood Sci Technol. 53(2):313–333. doi: 10.1007/s00226-018-1071-5.

Darband SG, Sadighparvar S, Yousefi B, Kaviani M, Ghaderi-Pakdel F, Mihanfar A, Rahimi Y, Mobaraki K, Majidinia M. 2020. Quercetin attenuated oxidative DNA damage through Nrf2 signaling pathway in rats with DMH induced colon carcinogenesis. Life Sci. 253:117584. doi: 10.1016/j.lfs.2020.117584.

Del Rio D, Rodriguez-Mateos A, Spencer JP, Tognolini M, Borges G, Crozier A. 2013. Dietary (poly)phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxid Redox Signal. 18(14):1818–1892. doi: 10.1089/ars.2012.4581.

De Santiago E, Gill CIR, Carafa I, Tuohy KM, De Peña M-P, Cid C. 2019. Digestion and colonic fermentation of raw and cooked *Opuntia ficus-indica* cladodes impacts bioaccessibility and bioactivity. J Agric Food Chem. 67(9):2490–2499. doi: 10.1021/acs.jafc.8b06480.

Di Lorenzo C, Colombo F, Biella S, Stockley C, Restani P. 2021. Polyphenols and human health: the role of bioavailability. Nutrients. 13(1):273. doi: 10.3390/nu13010273.

Diotallevi C, Fontana M, Latimer C, Ternan NG, Pourshahidi LK, Lawther R, O'Connor G, Conterno L, Gasperotti M, Angeli A, et al. 2021. Ex vivo faecal fermentation of human ileal fluid collected after wild strawberry consumption modulates human microbiome community structure and metabolic output and protects against DNA damage in colonic epithelial cells. Mol Nutr Food Res. 66(3):e2100405. doi: 10.1002/mnfr.202100405.

Dobani S, Latimer C, McDougall GJ, Allwood JW, Pereira-Caro G, Moreno-Rojas JM, Ternan NG, Pourshahidi LK, Lawther R, Tuohy KM, et al. 2021. Ex vivo fecal fermentation of human ileal fluid collected after raspberry consumption modifies (poly)phenolics and modulates geno-protective effects in colonic epithelial cells. Redox Biol. 40:101862. doi: 10.1016/j.redox.2021.101862.

Durazzo A, Lucarini M, Souto EB, Cicala C, Caiazzo E, Izzo AA, Novellino E, Santini A. 2019. Polyphenols: a concise overview on the chemistry, occurrence, and human health. Phytother Res. 33(9):2221–2243. doi: 10.1002/ptr.6419.

Falzone L, Candido S, Docea AO, Calina D. 2022. Editorial: inflammation and aging in chronic and degenerative diseases: current and future therapeutic strategies. Front Pharmacol. 13:1122786. doi: 10.3389/fphar.2022.1122786.

Gavrilas LI, Cruceriu D, Ionescu C, Miere D, Balacescu O. 2019. Pro-apoptotic genes as new targets for single and combinatorial treatments with resveratrol and curcumin in colorectal cancer. Food Funct. 10(6):3717–3726. doi: 10.1039/c9fo01014a.

Gómez de Cedrón M, Moreno Palomares R, Ramírez de Molina A. 2023. Metabolo-epigenetic interplay provides targeted nutritional interventions in chronic diseases and ageing. Front Oncol. 13:1169168. doi: 10.3389/fonc.2023.1169168.

Gupta D, Heinen CD. 2019. The mismatch repair-dependent DNA damage response: mechanisms and implications. DNA Repair. 78:60–69. doi: 10.1016/j.dnarep.2019.03.009.

Islam MA, Medha MM, Nahar AU, Al Fahad MA, Siraj MA, Seidel V. 2023. Cancer protective role of selected dietary polyphenols via modulating Keap1/Nrf2/ARE and interconnected signaling pathways. Nutr Cancer. 75(4):1065–1102. doi: 10.1080/01635581.2023.2183546.

Izquierdo-Vega JA, Morales-González JA, SánchezGutiérrez M, Betanzos-Cabrera G, Sosa-Delgado SM, Sumaya-Martínez MT, Morales-González Á, Paniagua-Pérez R, Madrigal-Bujaidar E, Madrigal-Santillán E. 2017. Evidence of some natural products with antigenotoxic effects. Part 1: fruits and polysaccharides. Nutrients. 9(2):102. doi: 10.3390/nu9020102.

Jin H, Tan X, Liu X, Ding Y. 2010. The study of effect of tea polyphenols on microsatellite instability colorectal cancer and its molecular mechanism. Int J Colorectal Dis. 25(12):1407–1415. doi: 10.1007/s00384-010-1047-x.

Kang KA, Piao MJ, Hyun YJ, Zhen AX, Cho SJ, Ahn MJ, Yi JM, Hyun JW. 2019. Luteolin promotes apoptotic cell death via upregulation of Nrf2 expression by DNA demethylase and the interaction of Nrf2 with p53 in human colon cancer cells. Exp Mol Med. 51(4):1–14. doi: 10.3390/molecules26134021.

Khan J, Deb PK, Priya S, Medina KD, Devi R, Walode SG, Rudrapal M. 2021. Dietary flavonoids: cardioprotective potential with antioxidant effects and their pharmacokinetic, toxicological and therapeutic concerns. Molecules. 26(13):4021. doi: 10.3390/molecules26134021.

Kostić K, Brborić J, Delogu G, Simić MR, Samardžić S, Maksimović Z, Dettori MA, Fabbri D, Kotur-Stevuljević J, Saso L. 2023. Antioxidant activity of natural phenols and derived hydroxylated biphenyls. Molecules. 28(6):2646. doi: 10.3390/molecules28062646.

Krawczyk M, Burzynska-Pedziwiatr I, Wozniak LA, Bukowiecka-Matusiak M. 2023. Impact of polyphenols on inflammatory and oxidative stress factors in diabetes mellitus: nutritional antioxidants and their application in improving antidiabetic therapy. Biomolecules. 13(9):1402. doi: 10.3390/biom13091402.

Lee U-J, Sohng JK, Kim B-G, Choi K-Y. 2023. Recent trends in the modification of polyphenolic compounds using hydroxylation and glycosylation. Curr Opin Biotechnol. 80:102914. doi: 10.1016/j.copbio.2023.102914.

Lessard-Lord J, Roussel C, Lupien-Meilleur J, Généreux P, Richard V, Guay V, Roy D, Desjardins Y. 2024. Short term supplementation with cranberry extract modulates gut microbiota in human and displays a bifidogenic effect. NPJ Biofilms Microbiomes. 10(1):18. doi: 10.1038/s41522-024-00493-w.

Li L, Li M, Chen Y, Yu Z, Cheng P, Yu Z, Cheng W, Zhang W, Wang Z, Gao X, et al. 2024. Function and therapeutic prospects of next-generation probiotic *Akkermansia muciniphila* in infectious diseases. Front Microbiol. 15:1354447. doi: 10.3389/fmicb.2024.1354447.

Lu Y, Shan S, Li H, Shi J, Zhang X, Li Z. 2018. Reversal effects of bound polyphenol from foxtail millet bran on multidrug resistance in human hct-8/fu colorectal cancer cell. J Agric Food Chem. 66(20):5190–5199. doi: 10.1021/acs.jafc.8b01659.

Luca SV, Macovei I, Bujor A, Miron A, Skalicka-Woźniak K, Aprotosoaie AC, Trifan A. 2020. Bioactivity of dietary polyphenols: the role of metabolites. Crit Rev Food Sci Nutr. 60(4):626–659. doi: 10.1080/10408398.2018.1546669.

Majidinia M, Bishayee A, Yousefi B. 2019. Polyphenols: major regulators of key components of DNA damage response in cancer. DNA Repair. 82:102679. doi: 10.1016/j.dnarep.2019.102679.

Maleki Dana P, Sadoughi F, Asemi Z, Yousefi B. 2022. The role of polyphenols in overcoming cancer drug resistance: a comprehensive review. Cell Mol Biol Lett. 27(1):1. doi: 10.1186/s11658-021-00301-9.

Masheghati F, Asgharzadeh MR, Jafari A, Masoudi N, Maleki-Kakelar H. 2024. The role of gut microbiota and probiotics in preventing, treating, and boosting the immune system in colorectal cancer. Life Sci. 344:122529. doi: 10.1016/j.lfs.2024.122529.

McDougall GJ, Allwood JW, Pereira-Caro G, Brown EM, Verrall S, Stewart D, Latimer C, McMullan G, Lawther R, O'Connor G, et al. 2017. Novel colon-available triterpenoids identified in raspberry fruits exhibit antigenotoxic activities in vitro. Mol Nutr Food Res. 61(2):1600327. doi: 10.1002/mnfr.201600327.

Mena P, Bresciani L, Brindani N, Ludwig IA, Pereira-Caro G, Angelino D, Llorach R, Calani L, Brighenti F, Clifford MN, et al. 2019. Phenyl-γ-valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: synthesis, analysis, bioavailability, and bioactivity. Nat Prod Rep. 36(5):714–752. doi: 10.1039/c8np00062j.

Moratilla-Rivera I, Sánchez M, Valdés-González JA, Gómez-Serranillos MP. 2023. Natural products as modulators of Nrf2 signaling pathway in neuroprotection. Int J Mol Sci. 24(4):3748. doi: 10.3390/ijms24043748.

Mou S, Zhou Z, He Y, Liu F, Gong L. 2017. Curcumin inhibits cell proliferation and promotes apoptosis of laryngeal cancer cells through Bcl-2 and PI3K/Akt, and by upregulating miR-15a. Oncol Lett. 14(4):4937–4942. doi: 10.3892/ol.2017.6739.

Moura C, Noguti J, Jesus G, Ribeiro F, Garcia F, Gollucke A, Aguiar O, Ribeiro D. 2013. Polyphenols as a chemopreventive agent in oral carcinogenesis: putative mechanisms of action using in-vitro and in-vivo test systems. Eur J Cancer Prev. 22(5):467–472. doi: 10.1097/CEJ.0b013e32835b6a94.

Niedernhofer LJ, Gurkar AU, Wang Y, Vijg J, Hoeijmakers JHJ, Robbins PD. 2018. Nuclear genomic instability and aging. Annu Rev Biochem. 87(1):295–322. doi: 10.1146/annurev-biochem-062917-012239.

Pan Y, Liu Z, Wang Y, Zhang L, Chua N, Dai L, Chen J, Ho CL. 2021. Evaluation of the anti-inflammatory and anti-oxidative effects of therapeutic human lactoferrin fragments. Front Bioeng Biotechnol. 9:779018. doi: 10.3389/fbioe.2021.779018.

Patel J, Baptiste BA, Kim E, Hussain M, Croteau DL, Bohr VA. 2020. DNA damage and mitochondria in cancer and aging. Carcinogenesis. 41(12):1625–1634. doi: 10.1093/carcin/bgaa114.

Patra S, Pradhan B, Nayak R, Behera C, Das S, Patra S, Efferth T, Jena M, Bhutia S. 2021. Dietary polyphenols in chemoprevention and synergistic effect in cancer: clinical evidences and molecular mechanisms of action. Phytomedicine. 90:153554. doi: 10.1016/j.phymed.2021.153554.

Pearce SC, Coia HG, Karl JP, Pantoja-Feliciano IG, Zachos NC, Racicot K. 2018. Intestinal in vitro and ex vivo models to study host-microbiome interactions and acute stressors. Front Physiol. 9:1584. doi: 10.3389/fphys.2018.01584.

Pereira-Caro G, Cáceres-Jimenez S, Bresciani L, Mena P, Almutairi TM, Dobani S, Pourshahidi LK, Gill CIR, Moreno Rojas JM, Clifford MN, et al. 2023. Excretion by subjects on a low (poly)phenol diet of phenolic gut microbiota catabolites sequestered in tissues or associated with catecholamines and surplus amino acids. Int J Food Sci Nutr. 74(4):532–543. doi: 10.1080/09637486.2023.2226369.

Prasad R, Katiyar SK. 2015. Polyphenols from green tea inhibit the growth of melanoma cells through inhibition of class I histone deacetylases and induction of DNA damage. Genes Cancer. 6(1-2):49–61. doi: 10.18632/genesandcancer.52.

Randah A, Daniel C, Rowland IR. 2018. Acai fruit as a source of bioactive phytochemicals. Nutrients. 10:496. doi: 10.17265/1934-7391/2016.08.005.

Rashid MH, Babu D, Siraki AG. 2021. Interactions of the antioxidant enzymes NAD(P)H: quinone oxidoreductase 1 (NQO1) and NRH: quinone oxidoreductase 2 (NQO2) with pharmacological agents, endogenous biochemicals and environmental contaminants. Chem Biol Interact. 345:109574. doi: 10.1016/j.cbi.2021.109574.

Rashid S, Freitas MO, Cucchi D, Bridge G, Yao Z, Gay L, Williams M, Wang J, Suraweera N, Silver A, et al. 2019. MLH1 deficiency leads to deregulated mitochondrial metabolism. Cell Death Dis. 10(11):795. doi: 10.1038/s41419-019-2018-y.

Rodríguez-Daza MC, Pulido-Mateos EC, Lupien-Meilleur J, Guyonnet D, Desjardins Y, Roy D. 2021. Polyphenol-mediated gut microbiota modulation: toward prebiotics and further. Front Nutr. 8:689456. doi: 10.3389/fnut.2021.689456.

Rudrapal M, Khairnar SJ, Khan J, Dukhyil AB, Ansari MA, Alomary MN, Alshabrmi FM, Palai S, Deb PK, Devi R. 2022. Dietary polyphenols and their role in oxidative stress-induced human diseases: insights into protective effects, antioxidant potentials and mechanism(s) of action. Front Pharmacol. 13:806470. doi: 10.3389/fphar.2022.806470.

Rufino AT, Costa VM, Carvalho F. 2021, Fernandes E. 2021. Flavonoids as antiobesity agents: a review. Med Res Rev. 41(1):556–585. doi: 10.1002/med.21740.

Saha S, Buttari B, Panieri E, Profumo E, Saso L. 2020. An overview of Nrf2 signaling pathway and its role in inflammation. Molecules. 25(22):5474. doi: 10.3390/molecules25225474.

Schmidlin CJ, Dodson MB, Madhavan L, Zhang DD. 2019. Redox regulation by Nrf2 in aging and disease. Free Radic Biol Med. 134:702–707. doi: 10.1016/j.freeradbiomed.2019.01.016.

Schumacher B, Pothof J, Vijg J, Hoeijmakers JHJ. 2021. The central role of DNA damage in the ageing process. Nature. 592(7856):695–703. doi: 10.1038/s41586-021-03307-7.

Silva MM, Rocha CRR, Kinker GS, Pelegrini AL, Menck CFM. 2019. The balance between Nrf2/GSH antioxidant mediated pathway and DNA repair modulates cisplatin resistance in lung cancer cells. Sci Rep. 9(1):17639. doi: 10.1038/s41598-019-54065-6.

Song M, Lee D, Chun K, Kim E. 2021. The role of Nrf2/Keap1 signaling pathway in cancer metabolism. Int J Mol Sci. 22(9):4376. doi: 10.3390/ijms22094376.

Soria-Valles C, López-Soto A, Osorio FG, López-Otín C. 2017. Immune and inflammatory responses to DNA damage in cancer and aging. Mech Ageing Dev. 165(Pt A):10–16. doi: 10.1016/j.mad.2016.10.004.

Telkoparan-Akillilar P, Panieri E, Cevik D, Suzen S, Saso L. 2021. Therapeutic targeting of the Nrf2 signaling pathway in cancer. Molecules. 26(5):1417. doi: 10.3390/molecules26051417.

Teng H, Fang T, Lin Q, Song H, Liu B, Chen L. 2017. Red raspberry and its anthocyanins: bioactivity beyond antioxidant capacity. Trends Food Sci Technol. 66:153–165. doi: 10.1016/j.tifs.2017.05.015.

Umeno A, Horie M, Murotomi K, Nakajima Y, Yoshida Y. 2016. Antioxidative and antidiabetic effects of natural polyphenols and isoflavones. Molecules. 21(6):708. doi: 10.3390/molecules21060708.

Wang M, Yu F, Zhang Y, Chang W, Zhou M. 2022. The effects and mechanisms of flavonoids on cancer prevention and therapy: focus on gut microbiota. Int J Biol Sci. 18(4):1451–1475. doi: 10.7150/ijbs.68170.

Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. 2012. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinform. 13(1):134. doi: 10.1186/1471-2105-13-134.

Yoshimura H, Yoshida H, Matsuda S, Ryoke T, Ohta K, Ohmori M, Yamamoto S, Kiyoshima T, Kobayashi M, Sano K. 2016. The therapeutic potential of epigallocatechin 3 gallate against human oral squamous cell carcinoma through inhibition of cell proliferation and induction of apoptosis: in vitro and in vivo murine xenograft study. Mol Med Rep. 20(2):1139–1148. doi: 10.3892/mmr.2019.10331.

Yu J, Liu T, Gao Z, Liu R, Wang Z, Chen Y, Cao J, Dong Y. 2022. *Akkermansia muciniphila* colonization alleviating high fructose and restraint stress-induced jejunal mucosal barrier disruption. Nutrients. 14(15):3164. doi: 10.3390/nu14153164.

Zakłos-Szyda M, Pawlik N, Polka D, Nowak A, Koziołkiewicz M, Podsędek A. 2019. *Viburnum opulus* fruit phenolic compounds as cytoprotective agents able to decrease free fatty acids and glucose uptake by caco-2 cells. Antioxidants. 8(8):262. doi: 10.3390/antiox8080262.

Zamora-Ros R, Knaze V, Rothwell JA, Hémon B, Moskal A, Overvad K, Tjønneland A, Kyrø C, Fagherazzi G, Boutron-Ruault M, et al. 2016. Dietary polyphenol intake in Europe: the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Eur J Nutr. 55(4):1359–1375. doi: 10.1007/s00394-015-0950-x.

Zhang A, Suzuki T, Adachi S, Naganuma E, Suzuki N, Hosoya T, Itoh K, Sporn MB, Yamamoto M. 2021. Distinct regulations of HO-1 gene expression for stress response and substrate induction. Mol Cell Biol. 41(11):e0023621. doi: 10.1128/MCB.00236-21.

Zhao LY, Mei JX, Yu G, Lei L, Zhang WH, Liu K, Chen XL, Kołat D, Yang K, Hu JK. 2023. Role of the gut microbiota in anticancer therapy: from molecular mechanisms to clinical applications. Signal Transduct Target Ther. 8(1):201. doi: 10.1038/s41392-023-01406-7.

Zhao Y, Jiang Q. 2021. Roles of the polyphenol-gut microbiota interaction in alleviating colitis and preventing colitis-associated colorectal cancer. Adv Nutr. 12(2):546–565. doi: 10.1093/advances/nmaa104.

Zhou Y, Jiang Z, Lu H, Xu Z, Tong R, Shi J, Jia G. 2019. Recent advances of natural polyphenols activators for keap1-Nrf2 signaling pathway. Chem Biodivers. 16(11):e1900400. doi: 10.1002/cbdv.201900400.

Zoetemelk M, Rausch M, Colin DJ, Dormond O, Nowak-Sliwinska P. 2019. Short-term 3d culture systems of various complexity for treatment optimization of colorectal carcinoma. Sci Rep. 9(1):7103. doi: 10.3389/fphys.2018.01584.