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Abstract

Irinotecan (CPT-11) is one of the main agents used to treat colorectal cancer; unfortunately, it is associated with increased intestinal mucositis developing. Luteolin has been shown to prevent damage induced by this chemotherapeutic in mice; thus, in this research, we have investigated luteolin's action mechanism in human intestinal epithelial cells. The potential of luteolin in reducing inflammation and oxidative stress induced by irinotecan in Caco-2 cells was evaluated by PCR through mRNA expression of inflammatory and oxidative genes and by ELISA at the protein level. To assess whether luteolin's ability to control irinotecan-induced damage occurs in a PPARγ dependent manner, experiments were performed on PPARγ downregulated cells. Irinotecan downregulated PPARγ expression and upregulated inflammatory and oxidative genes, while luteolin upregulated PPARγ, HO-1, SOD and decreased expression of IL-1β and iNOS. Interestingly, when the cells were co-stimulated with luteolin and irinotecan, the flavonoid reversed the inflammation and oxidative imbalance evoked by the chemotherapeutic. However, when these experiments were performed in cells downregulated for PPARγ, luteolin lost the capacity to increase PPARγ and reverse the effect of irinotecan in all tested genes, except by IL-1β. The present study showed that the protective effect of luteolin against irinotecan is PPARγ dependent.

Keywords: Intestinal mucositis, Caco-2, inflammation, oxidative stress, chemotherapy, rosiglitazone.

Abbreviations

CPT-11, Irinotecan; COX, Cyclooxygenase; CTRL; Control; DAMP, damage-associated molecular patterns; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethylsulfoxide; DPPH, 2,2- diphenyl-1- picrylhydrazyl; GPX, glutathione peroxidase; HO-1, heme oxygenase-1; IL, Interleukin; iNOS, inducible nitric oxide synthase; NQO-1, NAD(P)H quinone oxidoreductase 1; NRF-2, factor erythroid 2-related factor; PGE2, prostaglandin E2; PPARγ, peroxisome proliferator-activated receptor-gamma; ROS, reactive oxygen species; ShPPARγ, Caco-2 cell line knockdown for PPARγ; SOD, superoxide dismutase; TNF-α, tumor necrosis factor-alpha.

1 Introduction

Irinotecan (CPT-11), a chemotherapeutic drug analogous to camptothecin, is one of the leading agents used in treating colorectal cancer, acting inhibiting topoisomerase I [1]. Unfortunately, incorporating irinotecan into anticancer regimens is particularly associated with an increased risk of developing intestinal mucositis [2].

Intestinal mucositis is characterized by the mucosal barrier breakdown resulting in severe ulceration of the gastrointestinal tract and bacteria passing into the systemic circulation, increasing the risk of infections [3]. Sonis [4] has proposed that DNA damage, non-DNA damage, and ROS generation initiate an interesting and complex series of events that are still being defined, but that results in the activation of several transduction pathways resulting in the upregulation of up to 200 genes, many of which potentially influence mucosal toxicity.

Patients experiencing intestinal mucositis have nausea, vomiting, bleeding, abdominal pain, malnutrition, infections, sepsis, and diarrhea [5]. In fact, diarrhea is the main cause of patients' morbidity and mortality [6]. Currently, the treatments for mucositis are limited and largely target to oral rehydration and electrolyte replacement, as well as the use of pharmacologic agents to reduce fluid loss or decrease intestinal motility [3]; however, these approaches have low efficacy often leading to reduction of doses or interruption of the chemotherapeutic regime, consequently decreasing the chances of cancer remission [1].

In this way, searching for new therapeutic alternatives, we have previously evaluated the effect of the flavonoid luteolin in the prevention of irinotecan-induced intestinal mucositis in mice, evidencing that luteolin decreases oxidative stress, inflammatory process and maintains mucosal protective factors, such as mucus and expression of tight junctions, without interfering with the chemotherapeutic efficiency[7]. In the referenced study, it was observed that luteolin prevents the increase of cytokines such as TNF, IL-1β, and IL-6, without reducing PGE2 levels, in addition to ameliorating oxidative imbalance. This suggests that the modulation of transcription factors may be involved in the compound's mode of action, whereas modulation of COX does not appear to be part of the effect.

Actually, luteolin (3′,4′,5,7-tetrahydroxy flavone) is a flavone naturally found in several plant species, including broccoli, pepper, thyme, and celery [8,9], which exhibits a large number of biological activities reported in the literature and varied mechanisms of action described [10–16]. Among so many hypotheses, we call attention to the various reports suggesting that luteolin acts by activating the PPARγ pathway [17–20], which could

justify our findings mentioned before. Thus, in this study, we have developed an *in vitro* model of irinotecan-induced damage in human intestinal epithelial cells and provide evidence that this flavonoid's effect on the attenuation of cellular damage induced by irinotecan is dependent on the PPARγ pathway.

2. Methods

3. Materials

Luteolin (≥98% purity, powder) was commercially obtained from Active-Pharmaceutica (Palhoça, SC, Brazil). All other drugs and reagents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

3.1 Cell culture

Human intestinal epithelial cell line Caco-2 (ATCC® CRL-2102TM) were grown in Dulbecco's Modified Eagle's Medium (DMEM, Invitrogen, Life Technologies, Cergy-Pontoise, France) supplemented with 20% fetal calf serum (FCS, Dutscher, Brumath, France), 1% penicillin-streptomycin (Invitrogen, Life technologies), and 1% non-essential amino acids (Invitrogen, Life technologies).

All cell lines were cultured as confluent monolayers at 37°C in a controlled, 5% CO2 atmosphere.

3.1.2 Generation of PPARγ knockdown cells

Generation of PPARγ knockdown Caco-2 cells and the analysis of silencing of PPARγ expression by quantitative reverse transcription PCR and western-blot have been previously described [21].

3.2 Experimental Design

Caco-2 cells were seeded in 12-well plates (0.5×10^6). To synchronize the cell cycle, a medium deprived of serum was used 16 h before stimulation. Firstly, cells were incubated with irinotecan (10, 30, and 100 μM; Trebyxan® Laboratório Químico Farmacêutico Bergamo Ltda, Brazil) to determine the appropriate concentration to induce an inflammatory and oxidative response. After, the effect of luteolin (98% purity, powder from Active-pharmaceutica Palhoça, SC, Brazil) was standardized in three different concentrations (3, 10, and 30 μM). Rosiglitazone (1 μM; Sigma-Aldrich) was also incubated to visualize the effect of a full PPARγ agonist [22] in these cells. When necessary, the DMSO vehicle (Sigma-Aldrich) was used as control.

In another set of experiments, cells were incubated with Luteolin (3, 10, and 30 μM), or Rosiglitazone (1 μM) with irinotecan (100 μM) at the same time for 24 hours. Thus, the supernatant was collected for the quantification of cytokines using ELISA kits. The cells were subsequently washed with sterile PBS and lysed for RNA extraction, or another dosage described below.

Cell stimulations were performed in 3 or 6 replicates.

3.4 Enzyme-Linked Immunosorbent Assay

The supernatant of cells was used to quantify the cytokines TNF-α, IL-33, and IL-1β and the results were expressed as pg/ml. Total ROS and SOD-1 were measured on the

cell lysed. These results were expressed as pg/ml according to the protein level measured by the Bradford method.

ELISA kits from BD Biosciences (Franklin Lakes, New Jersey, USA) were used according to the manufacturer's instructions.

ROS

Total reactive oxygen species (ROS) were evaluated using a ROS assay Kit (Invitrogen, Thermo Fisher Scientific Logo) according to the manufacturer's instructions on the cultured cell supernatant and lysed cells.

3.5 RNA extraction

Firstly, cells were lysed by incubation in a solution containing large amounts of chaotropic ions. This lysis buffer immediately inactivated RNases, and total RNA was extracted with a Nucleospin RNA kit (Macherey-Nagel, Hoerdt, France). After RNAse inactivation, the total RNA was cleaned of traces of genomic DNA with a rDNase solution. The subsequent washing steps with different buffers removed salts, metabolites, and macromolecular cellular components, and then, pure RNA was finally eluted with RNase-free H_2O.

The RNA's purity was evaluated by UV spectroscopy on a Nanodrop system from 220 to 350 nm.

3.6 Quantitative RT-PCR

158 To performed quantitative reverse transcription-polymerase chain reaction (RT-
159 PCR), 1 μg of total RNA was reverse-transcribed into cDNA using the High-Capacity
160 cDNA Archive kit (Applied Biosystems).

161 Then, 2.5 μL of a 1:5 dilution of cDNA was employed for qPCR. ABI PRISM
162 StepOnePlus detection system (Applied Biosystem) using Power SYBR® Green PCR
163 master Mix (Applied Biosystem) was employed. Primer pairs were chosen with qPrimer
164 depot software according to table 1. Quantification of qPCR signals was performed using
165 ΔCt relative quantification method using GAPDH as a reference gene.

166

167 **Table 1.** Oligonucleotide Sequences for Quantitative RT-PCR

Genes	Forward sequences	Reverse sequences
GAPDH	5'-GACACCCACTCCTCCACCTTT-3'	5'-TTGCTGTAGCCAAATTCGTTGT-3'
PPARγ	5'-GCTGTCATTATTCTCAGTGGAGAC-3'	5'-GTCTTCTTGATCACATGCAGTAG-3'
IL-1β	5'- GATGCACCTGTACGATCACT - 3'	5'- GACATGGAGAACACCACTTG -3'
IL-33	5′-ACAGAATACTGAAAAATGAAGCC-3′	5′-CTTCTCCAGTGGTAGCATTTG-3′
iNOS	5'-CGGTGCTGTATTTCCTTACGAGGC GAAGAAGG -3'	5'-GGTGCTGCTTGTTAGGAGGTCAAGT AAAGGGC-3'
TNFα	5'-ATCAATCGGCCCGACTATCTC-3'	5'-ACAGGGCAATGATCCCAAAGT-3'
GPX	5′-GTG6TTG-GCT-TTT-CCC-TGC-AA-3'	5′-ACA-GCA-TAT-GCA-AGG6CAG-ATA-3'
NQO-1	5′-TGA-AGA-AGA-AAG-GAT-GGG-AGG-3'	5′-AGG-GGG-AAC-TGG-Aat-ATC-AC-3'
NRF-2	5′-TCA-GCC-AGG-CCA-GCA-CAT-CC-3'	5′-TCT-GCG-CCA-AAA-GCT-GCA-TGC-3'
HO-1	5′-TTG-CCA-GTG-CCA-CCA-AGT-TC-3'	5′-TCA-GCA-GCT-CCT-GCA-ACT-CC-3'

SOD	5′-ACA-AAG-ATG-GTG-TGG-CCG-AT-3’	5′-TCT-GGA-TCT6TTA-GAA-ACC-GCG-A-3’

168
169

170 **3.7 Statistics**

171 The data were analyzed by an investigator blinded to the experimental conditions.
172 Each *in vitro* experiment was conducted at least three times independently. The
173 Kolmogorov-Smirnov normality test was applied to verify the data normality. The data
174 were expressed as mean ± SEM, and one- or two-way analysis of variance (ANOVA)
175 followed by Bonferroni's post hoc test was applied to verify the differences between means.
176 Statistical analysis was performed using the software GraphPad Prism
177 (RRID:SCR_002798) version 7.00 (GraphPad Software, La Jolla, CA, USA). A $p < 0.05$
178 was considered significant.

179

180 **4. Results**

181

182 **4.1 Irinotecan concentrations to induce intestinal cell damage**

183 Previously studies have demonstrated that irinotecan does not significantly reduce
184 the cell viability of Caco-2 cells at 1 to 100 μM in 24 hours of incubation [23]. In this way,
185 we have investigated the effect of irinotecan at 10, 30, and 100 μM during 24 hours of
186 incubation, evaluating different target genes in the human epithelial intestinal cells to select
187 the optimal concentration to maximize inflammation and oxidative stress without overly
188 affecting viability.

189

190 **4.2 Irinotecan-induced inflammation and oxidative imbalance in Caco-2 cells**

As shown in figure 1, irinotecan at 100 μM decreased in 60% the PPARγ gene expression compared to control, and significantly increased the expression of the cytokines interleukin (IL)-1β (9.97-folds); interleukin (IL)-33 (17.01-folds); tumor necrosis factor-alpha (TNF-α) (16.44-folds); and inducible nitric oxide synthase (iNOS) (8.51-folds).

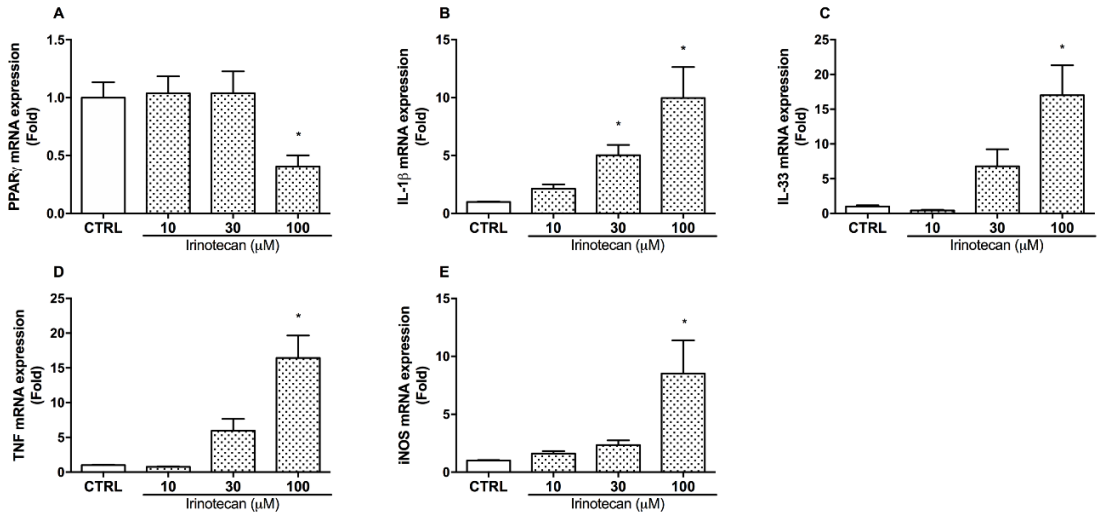
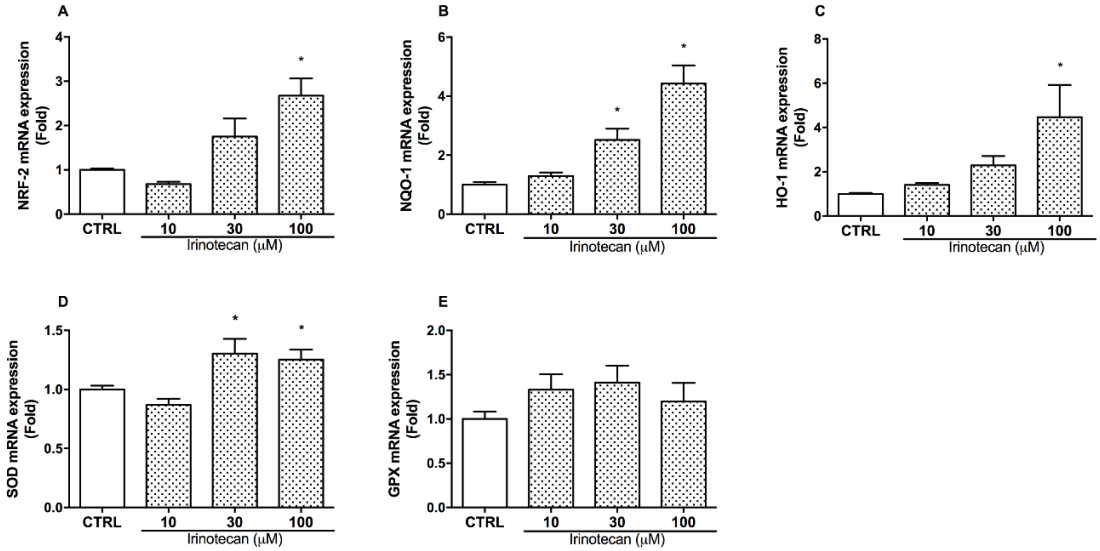


Fig 1. Effect of Irinotecan on peroxisome proliferator-activated receptor-gamma (PPARγ) (A) interleukin (IL)-1β (B); IL-33 (C); tumor necrosis factor-alpha (TNF-α) (D); and inducible nitric oxide synthase (iNOS) (E) expression. Cells were stimulated for 24 h with irinotecan. Results represent mean ± SEM (3) independent experiments in triplicate or sextuplicate, $9 < n < 12$) of the fold change of each gene expression normalized to GAPDH level. The expression level measured in control cells was used as a reference and defined as 1. * $p < 0.05$ compared to control (CTRL).

In figure 2, it is possible to observe that irinotecan upregulated the expression of factor erythroid 2-related factor (NRF-2 - 2.67-folds), NAD(P)H quinone oxidoreductase 1

(NQO-1 - 4.42-folds); heme oxygenase-1 (HO-1 - 4.46-folds), and superoxide dismutase (SOD - 1.25-folds), while it did not alter glutathione peroxidase mRNA expression



compared to control.

Fig 2. Effect of Irinotecan on nuclear factor erythroid 2-related factor (NRF-2) (A); NAD(P)H quinone oxidoreductase 1 (NQO-1) (B); heme oxygenase-1 (HO-1) (C); superoxide dismutase (SOD) (D); and glutathione peroxidase (GPX) (E) expression. Cells were stimulated for 24 h with irinotecan. Results represent mean ± SEM (3 independent experiments in triplicate or sextuplicate, $9 < n < 12$) of the fold change of each gene expression normalized to GAPDH level. The expression level measured in control cells was used as a reference and defined as 1. * $p < 0.05$ compared to control (CTRL).

4.3 Effect of luteolin on Caco-2 cells gene expression

As observed in the figure S2 (supplementary material), the cellular viability of Caco-2 cells incubated with luteolin at 1, 3, and 30 μM is more than 82%, therefore, the effect of luteolin on Caco-2 cells was evaluated at these three concentrations, as can be seen

in figure 3. The luteolin at 30 μM caused a significant increase in the PPARγ mRNA expression (6.29-folds) compared to the control. Moreover, the flavonoid reduced by 88% the expression of IL-1β and by 53% the iNOS expression, which was also significative reduced by rosiglitazone (1μM) incubation (38%). Luteolin did not induce any change in the expression of TNF-α. The IL-33 expression from cells incubated only with luteolin is not shown because the flavonoid-induced gene expression did not reach the cycle threshold.

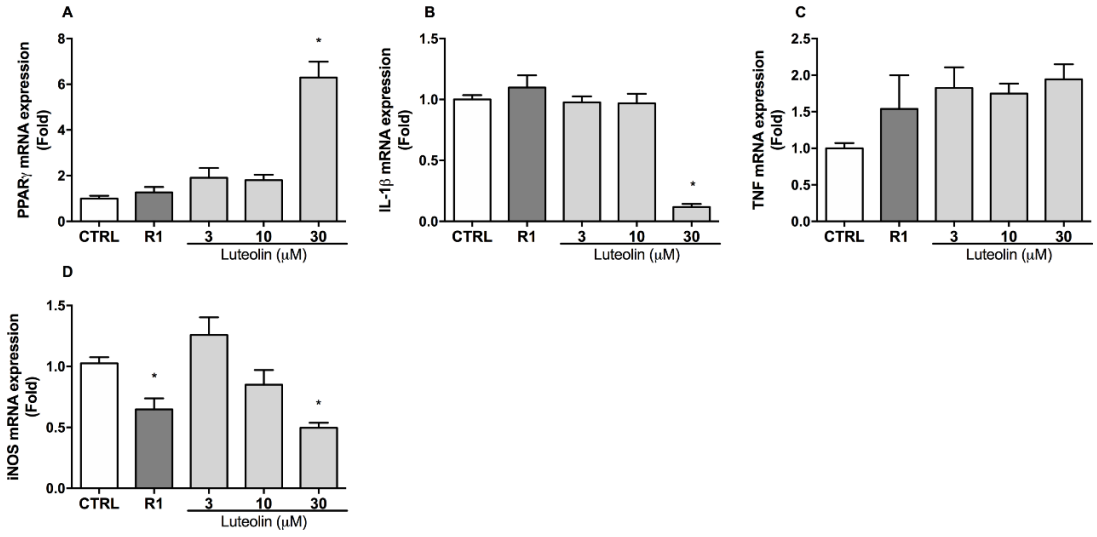


Fig 3 Effect of Luteolin on peroxisome proliferator-activated receptor-gamma (PPARγ) (A) interleukin (IL)-1β (B); tumor necrosis factor-alpha (TNF-α) (C); and inducible nitric oxide synthase (iNOS) (D) expression. Cells were stimulated for 24 h with luteolin or rosiglitazone 1μM (R1). Results represent mean ± SEM (3 independent experiments in triplicate or sextuplicate, $9 < n < 12$) of the fold change of each gene expression normalized to GAPDH level. The expression level measured in control cells was used as a reference and defined as 1. * $p < 0.05$ compared to control (CTRL).

Moreover, luteolin did not induce significative alteration on the NRF-2, NQO-1, and GPX mRNA expression, but led to a significative increase in the HO-1 (8.73-folds) and SOD (1.37-folds) expression, compared to control (Figure 4).

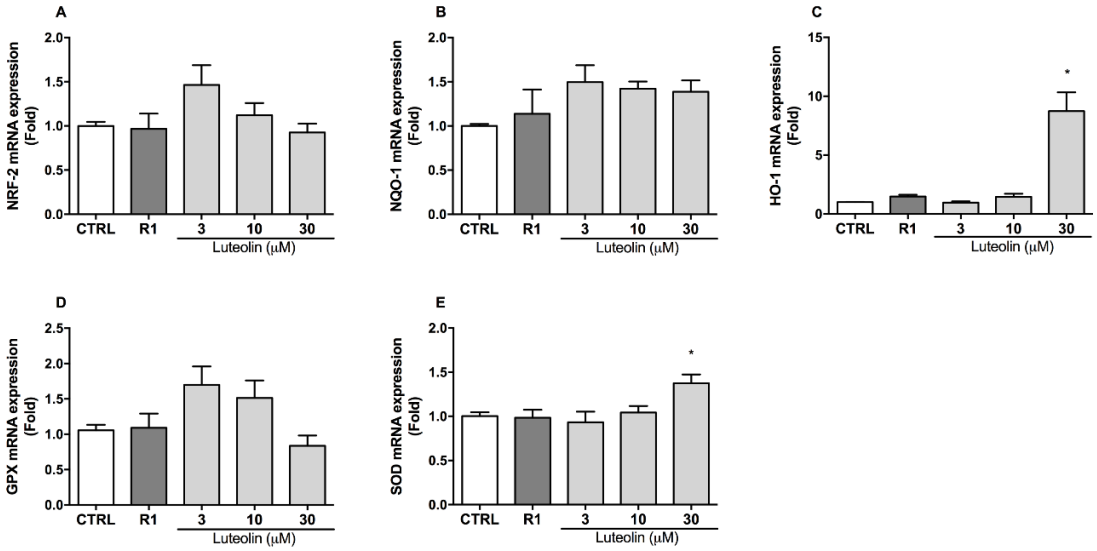


Fig 4. Effect of Luteolin on factor erythroid 2-related factor (NRF-2) (A); NAD(P)H quinone oxidoreductase 1 (NQO-1) (B); heme oxygenase-1 (HO-1) (C); superoxide dismutase (SOD) (D) and glutathione peroxidase (GPX) expression; (E). Cells were stimulated for 24 h with luteolin or rosiglitazone 1μM (R1). Results represent mean ± SEM (3 independent experiments in triplicate or sextuplicate, $9 < n < 12$) of the fold change of each gene expression normalized to GAPDH level. The expression level measured in control cells was used as a reference and defined as 1. * $p < 0.05$ compared to control (CTRL).

4.4 Luteolin inhibits damage induced by irinotecan on Caco-2 cells

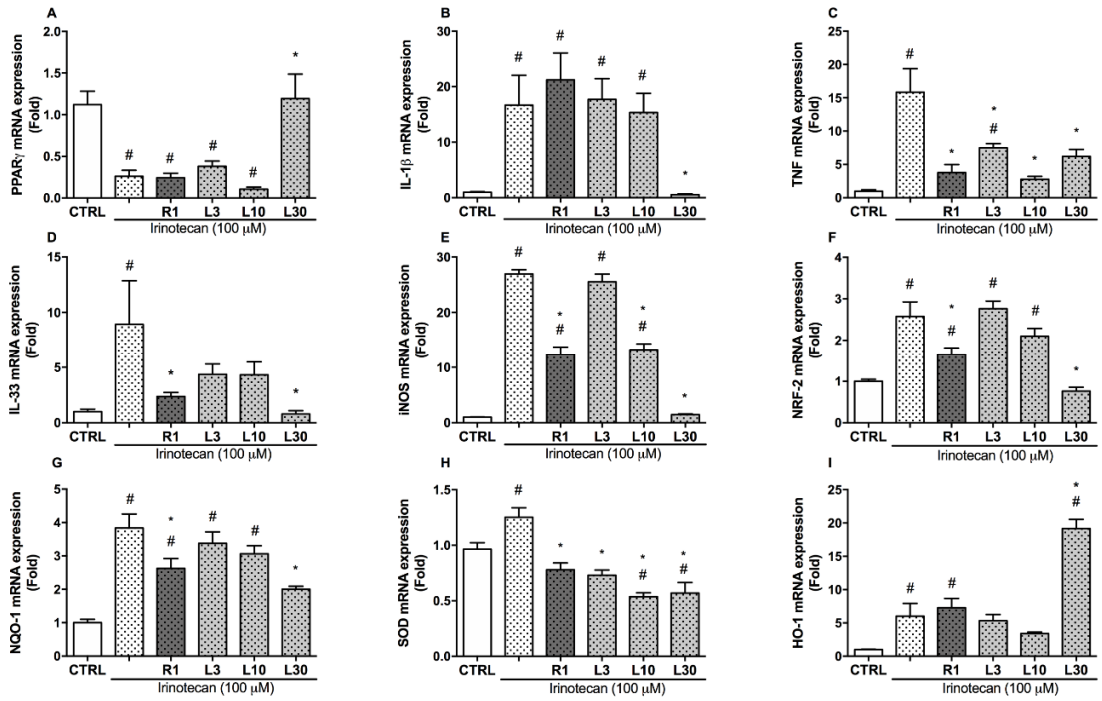
From the data obtained, we have selected the concentration of 100 μM of irinotecan to induce inflammatory and oxidative imbalance in the human intestinal epithelial cells.

Thus, we incubated the irinotecan with luteolin at the same time to evaluate the expression of genes that had been altered by the chemotherapeutic.

Interestingly, the PPARγ down-regulation induced by irinotecan was reversed by luteolin at 30 μM, reaching a mRNA expression similar to the control cells (Figure 5A). In accordance, the IL-1β up-regulation induced by irinotecan was significantly reduced by luteolin (96%), as well as the expression of TNF-α (60%), IL-33 (91%), iNOS (94%) (Figure 5 B, C, D, and E).

Besides, luteolin reversed the oxidative imbalance evoked by the chemotherapy, restoring expression of NRF-2, and decreasing the alteration produced in NQO-1 and SOD (Figure 5 F, G, and H). Otherwise, the HO-1 expression increased by luteolin itself (Figure 4C), was even more increased by the co-incubation of luteolin and irinotecan (Figure 5 I).

The PPARγ agonist rosiglitazone (1 μM) was able to reverse the damage induced by irinotecan in the TNF-α, IL-33, iNOS, NRF-2, NQO-1and SOD expression (Figure 5 C, D,



E, F, G, H).

Fig 5. Effect of Luteolin against gene expression disturbance induced by irinotecan. Peroxisome proliferator-activated receptor-gamma (PPARγ) (A) interleukin (IL)-1β (B); tumor necrosis factor-alpha (TNF-α) (C); IL-33 (D); inducible nitric oxide synthase (iNOS) (E); factor erythroid 2-related factor (NRF-2) (F); NAD(P)H quinone oxidoreductase 1 (NQO-1) (G); superoxide dismutase (SOD) (H); and heme oxygenase-1 (HO-1) (I) expression. Cells were stimulated for 24 h with luteolin 30μM (L30); rosiglitazone 1μM (R1) or L30 and R1 plus irinotecan 100 μM. Results represent mean ± SEM (3) independent experiments in triplicate or sextuplicate, $9 < n < 12$) of the fold change of each gene expression normalized to GAPDH level. The expression level measured in control cells was used as a reference and defined as 1. # $p < 0.05$ compared to control (CTRL); * $p < 0.05$ compared to irinotecan.

Besides the gene expression modulation, we measured the levels of secreted cytokines by Elisa. Irinotecan increased the levels of IL-β (1.56-folds), IL-33 (1.43-folds), and TNF-α (2.30-folds). Following the data observed at mRNA, cells incubated with luteolin 30 μM decreased by 48%, 44%, 43% the levels of the respective interleukins. Although luteolin incubation did not significantly decrease TNF-α levels compared to irinotecan, it was not significantly increased compared to basal (Figure 6 A, B, and C).

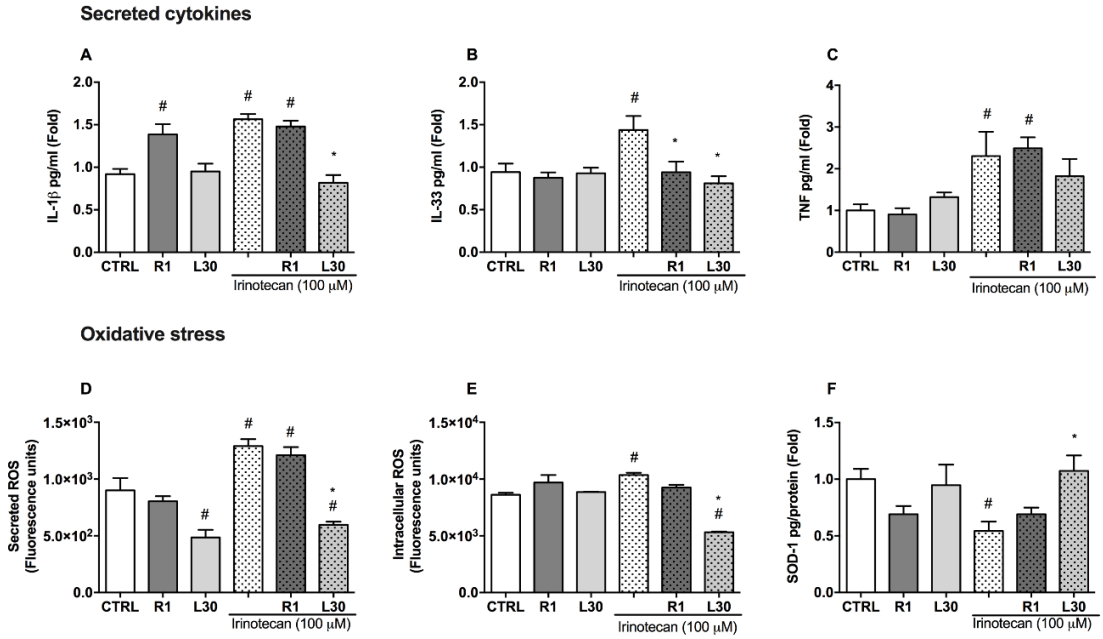


Fig 6. Effect of Luteolin against inflammation and oxidative stress induced by irinotecan. Interleukin (IL)-1β (A); IL-33 (B); TNF-α (C) and reactive oxygen species (ROS) amount (D) were measured by Elisa in the supernatant of cells stimulated for 24 h with luteolin 30μM (L30); rosiglitazone 1μM (R1) or L30 and R1 plus irinotecan 100 μM. ROS (E) and the levels of Superoxide Dismutase 1 (SOD-1) (F) were measured on the cell lysed. Results represent mean ± SEM (3 independent experiments in triplicate or sextuplicate, $9 < n < 12$). # $p < 0.05$ compared to control (CTRL); * $p < 0.05$ compared to irinotecan.

Moreover, total ROS present in the culture medium and the supernatant of the cell lysate were increased by irinotecan compared to control (Figure 6 D and E). In contrast, in the luteolin-incubated samples, it was significative reduced. Interestingly, the levels of SOD-1 were decreased by irinotecan at the protein level and reversed by luteolin (Figure 6 F).

298

299 **4.5 Effect of luteolin is dependent on PPARγ**

300 To identify if luteolin effects depend on PPARγ, we have investigated the mRNA
301 expression of some genes altered by irinotecan in the previous data in PPARγ knockdown
302 Caco-2 cells. To this end, we used a Caco-2 shPPARγ cell line that stably expresses a short
303 hairpin anti-sense RNA against PPARγ, leading to specific downregulation of PPARγ [21]
304 and Caco-2 shLUC as control cells (cells expressing a control shRNA directed against the
305 luciferase gene). Compared to control cells, the expression of PPARγ in Caco-2 ShPPARγ
306 cell line was significative reduced (Figure 7 A).

307 Interestingly, luteolin reversed the effect of irinotecan by decreasing PPARγ in
308 control cells shLUC, but completely lost the capacity to increase the gene expression alone
309 and reverse the gene downregulation induced by irinotecan in the cells shPPARγ (Figure 7
310 B). Moreover, luteolin and rosiglitazone showed decreased TNF-α, NQO-1, NRF-2, and
311 SOD mRNA expression compared to irinotecan in the shLUC cells, but the same effects
312 were not observed in the cells shPPARγ (Figure 7 D, E, F, and G). Conversely, the effect of
313 luteolin of reversing IL-1β upregulation induced by irinotecan was maintained even in the
314 PPARγ knockdown Caco-2 cells (Figure 7 C).

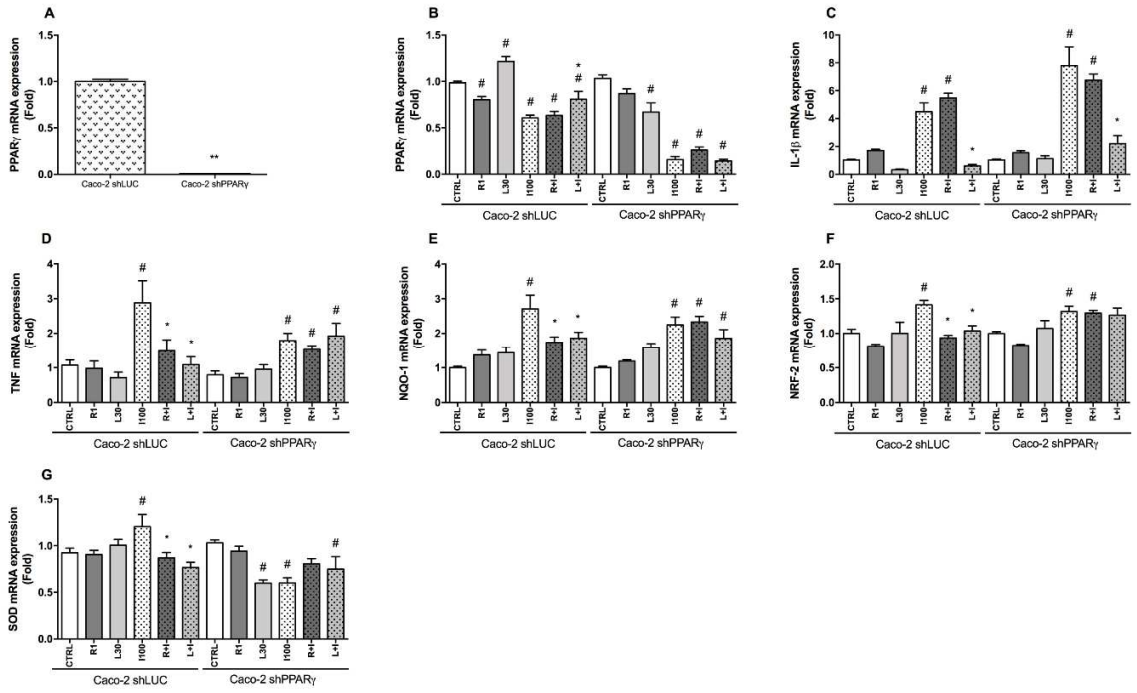


Fig 7. The effect of luteolin is strongly reduced in peroxisome proliferator-activated receptor-gamma (PPARγ) knockdown Caco-2 cells. Caco-2 cell line knockdown for PPARγ (ShPPARγ) expressed significantly fewer PPARγ expression compared to control cells (ShLuc) (A). The expression level measured in ShLuc cells (arbitrarily defined as one) was used as a reference. The results represent a triplicate of the same clone of ShLuc and ShPPARγ Caco-2 cells, respectively. The cells were stimulated for 24 h with luteolin 30 μM (L30); rosiglitazone 1μM (R1); irinotecan 100 μM (I100); or L30 and R1 plus irinotecan 100 μM (L+I and R+I, respectively). Peroxisome proliferator-activated receptor-gamma (PPARγ) (B) interleukin (IL)-1β (C); tumor necrosis factor-alpha (TNF-α) (D); NAD(P)H quinone oxidoreductase 1 (NQO-1) (E); factor erythroid 2-related factor (NRF-2) (F); and superoxide dismutase (SOD) (G) expression. Results represent mean ± SEM (3) independent experiments in triplicate or sextuplicate, $9 < n < 12$) of the fold change of each gene expression normalized to GAPDH level. The expression level measured in control

329 cells was used as a reference and defined as 1. # $p < 0.05$ compared to control (CTRL); * p
330 < 0.05 compared to irinotecan.
331
332 **5. Discussion**
333 Irinotecan-induced intestinal mucositis produces mucosal changes associated with
334 epithelial vacuolation, goblet cell hyperplasia, villous shortening, crypt cell apoptosis, and
335 infiltration of leukocytes into the lamina propria [24]. Several lines of evidence have
336 demonstrated that these changes appear to be related to specific inflammatory mediators
337 that are crucial factors contributing to the pathogenesis of intestinal mucositis [1], as well
338 as the reactive oxygen species generation [4]. Therefore, this research focused on
339 evaluating gene expression of inflammatory and oxidative related genes in Caco-2-
340 enterocytes exposed to irinotecan, subsequently evaluating the PPARγ dependent effect of
341 luteolin on the attenuation of irinotecan-induced disorders.
342 Kontos et al. [23], have shown that the cell viability of Caco-2 cells incubated with
343 irinotecan (1-100 μM) for 24 hours is more than 80%. In this way, to select the optimal
344 concentration to maximize inflammation and oxidative stress, we have incubated cells with
345 3 to 100 μM of the chemotherapeutic, thus, selecting the higher concentration to continue
346 the study since it induced changes in most of the evaluated genes. Caco-2 cells have been
347 used to study methotrexate [25,26] and 5-fluoracil induced- mucositis [27], but as far as we
348 know, this is the first study proposing an *in vitro* model for the study of cell damage
349 induced by irinotecan, that resembles the *in vivo* intestinal mucositis. Therefore, we have
350 first investigated the chemotherapeutic effect in different target genes involved in the
351 intestinal mucositis process.

The data obtained showed that irinotecan upregulated the expression of IL-1β, TNF-α, IL-33, and iNOS. It is described that damage induced during intestinal mucositis results in the activation of transductions pathways, of which the NFκB-mediated inflammatory pathway plays an important role in mucosal injury [5,28], resulting in the production of pro-inflammatory cytokines, including TNF-α, IL-1β [4]. Moreover, Guabiraba et al. [29] reported that irinotecan induces direct epithelial cell damage by modulating the release of IL-33 and Lima et al. [30] have described that irinotecan increases immunoexpression of iNOS.

Besides, the results presented herein showed that irinotecan upregulated NRF-2 mRNA expression in the Caco-2 cells and the related genes NQO-1, HO-1, SOD and even caused a significant increase in reactive species of oxygen (ROS) (Figure E and F) into the cells. In fact, the increased ROS might lead to lipid peroxidation of cell-membrane-bound molecules, resulting in the upregulation of NRF2 [31]. This transcription factor is a key player in the cellular stress response, binding into cis-acting elements in the promoters of target genes; it encodes a series of cytoprotective proteins, including NAD(P)H:quinone oxidoreductase (NQO-1), heme oxygenase 1 (HO-1) and superoxide dismutase (SOD) [32,33].

Interestingly, irinotecan significantly decreased PPARγ mRNA expression. The peroxisome proliferator-activated receptor-gamma belongs to the nuclear receptor superfamily of ligand-activated transcriptional factors, which controls genes involved in cell differentiation, control of glucose homeostasis, and lipid metabolism [34]. Besides adipocytes, the other major tissue expressing PPARγ is the intestine [35,36], linked to the modulation of immune and inflammatory response. Numerous studies have suggested the

375 therapeutic potential of targeting PPARγ to treat inflammatory bowel diseases, such as
376 colitis [37], and the downregulation of PPARγ induced by irinotecan demonstrated in this
377 work reinforces that this could be a new strategy in the management of intestinal mucositis
378 which has been little explored so far.

379 Luteolin (Figure S1, supplementary material) is a naturally occurring flavonoid
380 described as a PPARγ partial agonist [17] and proved to attenuate intestinal mucositis
381 irinotecan induced in mice [7]. Interestingly, the intestinal cells stimulated with luteolin at
382 30 μM showed increased mRNA expression of PPARγ, as well as Ding et al. [38] have
383 found that luteolin treatment (20 μmol/L) increases expression and transcriptional
384 activation of PPARγ and its target genes adiponectin, leptin, and GLUT4 in 3T3-L1
385 adipocytes.

386 Moreover, luteolin stimulation enhanced HO-1 and SOD expression. Indeed,
387 Polvani et al. [39] have described that PPARγ induces HO-1 expression in human vascular
388 cells. These findings highlight the antioxidative potential of luteolin, since SOD is
389 responsible for the catalysis of superoxide to hydrogen peroxide and has also been
390 implicated in diverse roles in the cell, including that of a transcription factor [40], while
391 heme oxygenases catalyze the degradation of heme to biliverdin and are related to the
392 reduction of oxidative stress, diminished inflammatory response, and decreased rate of
393 apoptosis [41].

394 Additionally, luteolin decreased L-1β and iNOS mRNA levels, similar to those
395 found by other authors in different types of cells [16]. Although rosiglitazone is a known
396 full PPARγ agonist, it did not induce the same response as luteolin in the target genes
397 evaluated. Puhl et al. [17] have shown that luteolin acts as a potent anti-inflammatory agent

398 through PPARγ in HCECs, but exhibits weak partial agonist behavior relative to the full
399 agonist rosiglitazone in cell transactivation assays, probably, the different ways in which
400 the ligands bind in the PPARγ receptor are responsible for the different responses of them.
401 Further, it is worth noting that natural partial agonists, when compared to full synthetic
402 agonists thiazolidinediones lead to slighter side effects [42].

403 Moreover, when the cells were co-stimulated with irinotecan and rosiglitazone, the
404 PPARγ agonist was able to reverse the changes induced by the chemotherapeutic in the
405 mRNA expression of TNF-α, IL-33, iNOS, NRF-2, NQO-1, and SOD, as well as luteolin
406 30 μM, that additionally reversed the changes in PPARγ, and IL-1β expression. The effect
407 of the flavonoid in attenuating intestinal damage induced by irinotecan was confirmed at
408 protein levels once the compound reversed the enhancement of IL-1β, and IL-33 generated
409 by irinotecan. Regarding TNF-α expression, the data showed that luteolin effectively
410 inhibited the irinotecan-induced upregulation, which is in agreement with the findings of
411 the previous *in vivo* experiment, where luteolin reversed the increase in this cytokine
412 induced by irinotecan in the duodenum of mice [7]. However, the results presented here
413 also showed that the flavonoid itself did not reduce cytokine mRNA expression, indicating
414 that TNF-α is regulated by the flavonoid when there is an aggressive stimulus, similarly to
415 what was observed for rosiglitazone. Although luteolin reversed the up-regulation of TNF-
416 α induced by irinotecan, only a slight decrease in the amount of cytokine secreted was
417 observed. The fact that TNF-α exists in two forms, a membrane-bound and a soluble form
418 [43] may explain this. Moreover, it is important to mention that the reduction in TNF-α
419 levels *in vivo* is more evident, probably because it involves not only cytokine secretion by
420 intestinal epithelial cells, but also by macrophages, lymphocytes, and neutrophils [43].

Conversely, the mRNA expression of SOD was increased by irinotecan and reversed by luteolin, but the cells incubated with irinotecan had decreased antigen level of SOD-1 detected by ELISA. Human SOD-1 is a polypeptide that forms a homodimer, with each monomer binding one copper and zinc ions within a disulfide-bonded conformer. The maturation of SOD-1 is dependent on a series of posttranslational modifications such as Zn(ii) and Cu(i) binding, disulfide bond formation, and dimerization. In contrast, the disruption of any of these steps results in an inactive protein [44]. Thus, irinotecan-induced NRF-2 pathway activation may have induced positive regulation of SOD mRNA, but protein maturation has not occurred, and antigen levels for this protein remained low. Then, with SOD diminished, oxidative stress becomes even more exacerbated, and intracellular ROS amount is increased in these cells. Besides, at present, three distinct isoforms of SOD have been identified in mammals, being SOD1 the most abundant enzyme found in the cytoplasm, nuclear compartments, and lysosomes of cells. In contrast, SOD2 has been localized to mitochondria and SOD3 has been detected in extracellular fluids [45], thereby, it is not possible to rule out the possibility that these two other protein isoforms are increased.

To this point, the results obtained in this study fomented our hypothesis that luteolin effects in attenuating damage irinotecan-induced are PPARγ depended. Thus, to confirm this theory, we investigated the mRNA expression of some genes altered by irinotecan in PPARγ knockdown Caco-2 cells. As expected, in the control cells (cells expressing a control shRNA directed against the luciferase gene - Caco-2 shLUC), the full PPARγ agonist rosiglitazone and the flavonoid luteolin were able to reverse the changes induced by

443 irinotecan in the mRNA expression of TNF-α, NRF-2, NQO-1, and SOD, however in
444 PPARγ knockdown cells (Caco-2 shPPARγ) both substances lost the activity.

445 In contrast, luteolin's effect on the mRNA expression of IL-1β was maintained in
446 the PPARγ knockdown cells. During intestinal mucositis, TLR-2 and TLR-9 are activated
447 by DAMPs and PAMPs in intestinal epithelial cells, activating the downstream cascade of
448 the TIR domain, the differentiation adaptor protein (MyD88), which induces signaling
449 pathways such as NFkB, IL-1, IL-18 [24], nevertheless, luteolin itself is a potent
450 antioxidant molecule (IC50 of ~1.84 μg/ml was found in DPPH assay) [7], then, its directly
451 scavenging properties on ROS formed during the pathophysiology of mucositis, at least in
452 part, contribute to less DAMPS generation, decreased activation of TLR and consequently
453 decrease induction of IL-1β expression, independently of PPARγ-pathway.

454 But still, the effects on mRNA expression of NRF-2, NQO-1, and SOD showed that
455 outside the directly scavenging effect, luteolin activity is PPARγ dependent; in fact, natural
456 ligands of PPARγ are produced during oxidative stress and PPARγ, if already expressed,
457 may be one of the first responders directly inducing an arsenal of antioxidant molecules,
458 inhibiting prooxidants and at the same time protecting the cells from apoptosis [39].

459 Furthermore, luteolin completely lost the capacity to increase PPARγ in knockdown
460 cells, confirming the PPARγ-dependent effect of flavonoid luteolin against the damage
461 induced by the chemotherapeutic. However, PPAR-γ ligands exert their anti-inflammatory
462 effects often triggering cross talks with other signaling pathways [46]; thus, it is important
463 to mention that PPARγ activation can also result in the NF-κB nuclear transcription factor
464 repression signaling by various proposed mechanisms [47], contributing to decrease the
465 transcription of inflammatory mediators. In addition, these cross talks with other signaling

pathways also may explain why luteolin displays concentration-dependently effects for some genes but not others.

Finally, with the data presented here, it is acceptable to assume that the mechanism underlying the effects of luteolin in attenuating irinotecan-induced intestinal cell damage involves its direct scavenge property and increase in the PPARγ expression, regulating inflammation and oxidative stress by controlling gene expression of cytokines and oxidative genes. In addition, together with our previous study carried out in mice, this study supports the production chain in the search for new drugs for the treatment of intestinal mucositis, collaborating with the pre-clinical validation of the product, which demonstrates potential to be evaluated in clinical trials.

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Author Contributions

Thaise Boeing, Silvia Speca, and Anthony Martin Mena carried out the experimental work. Thaise Boeing, Silvia Speca, Priscila de Souza, Luisa Mota da Silva, and Sérgio Faloni de Andrade analyzed the data, wrote and corrected the manuscript. Laurent Dubuqoy, Benjamin Bertin, Pierre Desreumax, performed the study's design and

contributed with reagents and analytical tools. All authors have read and approved the manuscript.

Declaration of competing interest

The authors declare no conflicts of interest.

References

[1] R.A. Ribeiro, C.W.S. Wanderley, D.V.T. Wong, J.M.S.C. Mota, C.A.V.G. Leite, M.H.L.P. Souza, F.Q. Cunha, R.C.P. Lima-Júnior, Irinotecan- and 5-fluorouracil-induced intestinal mucositis: insights into pathogenesis and therapeutic perspectives, Cancer Chemotherapy and Pharmacology. 78 (2016) 881–893. https://doi.org/10.1007/s00280-016-3139-y.

[2] J.A. Jones, E.B.C. Avritscher, C.D. Cooksley, M. Michelet, B.N. Bekele, L.S. Elting, Epidemiology of treatment-associated mucosal injury after treatment with newer regimens for lymphoma, breast, lung, or colorectal cancer, Supportive Care in Cancer. 14 (2006) 505–515. https://doi.org/10.1007/s00520-006-0055-4.

[3] Y.Z.A. Van Sebille, R. Stansborough, H.R. Wardill, E. Bateman, R.J. Gibson, D.M. Keefe, Management of Mucositis During Chemotherapy: From Pathophysiology to Pragmatic Therapeutics, Current Oncology Reports. 17 (2015). https://doi.org/10.1007/s11912-015-0474-9.

[4] S.T. Sonis, The Pathobiology of Mucositis, Journal of Neuropathology and Experimental Neurology. 4 (2004) 277–284. https://doi.org/10.1038/nrc1318.

[5] B. Vanhoecke, E. Bateman, B. Mayo, E. Vanlancker, A. Stringer, D. Thorpe, D. Keefe, Dark Agouti rat model of chemotherapy-induced mucositis: Establishment and current state of the art, Experimental Biology and Medicine. 240 (2015) 725–741. https://doi.org/10.1177/1535370215581309.

[6] J. Andreyev, P. Ross, C. Donnellan, E. Lennan, P. Leonard, C. Waters, L. Wedlake, J. Bridgewater, R. Glynne-Jones, W. Allum, I. Chau, R. Wilson, D. Ferry, Guidance on the management of diarrhoea during cancer chemotherapy, The Lancet Oncology. 15 (2014) e447–e460. https://doi.org/10.1016/S1470-2045(14)70006-3.

[7] T. Boeing, P. de Souza, S. Speca, L.B. Somensi, L.N.B. Mariano, B.J. Cury, M. Ferreira dos Anjos, N.L.M. Quintão, L. Dubuqoy, P. Desreumax, L.M. da Silva, S.F. de Andrade, Luteolin prevents irinotecan-induced intestinal mucositis in mice through antioxidant and anti-inflammatory properties, British Journal of Pharmacology. 177 (2020) 2393–2408. https://doi.org/10.1111/bph.14987.

[8] M. Lopez-Lazaro, Distribution and Biological Activities of the Flavonoid Luteolin, Mini-Reviews in Medicinal Chemistry. 9 (2009) 31–59. https://doi.org/10.2174/138955709787001712.

[9] K. Shimoi, H. Okada, M. Furugori, T. Goda, S. Takase, M. Suzuki, Y. Hara, H. Yamamoto, N. Kinae, Intestinal absorption of luteolin and luteolin 7- *O* -β-glucoside in rats and humans, FEBS Letters. 438 (1998) 220–224. https://doi.org/10.1016/S0014-5793(98)01304-0.

[10] G. Seelinger, I. Merfort, C.M. Schempp, Anti-oxidant, anti-inflammatory and anti-allergic activities of luteolin, Planta Medica. 74 (2008) 1667–1677. https://doi.org/10.1055/s-0028-1088314.

[11] P. Ashokkumar, G. Sudhandiran, Luteolin inhibits cell proliferation during Azoxymethane-induced experimental colon carcinogenesis via Wnt/ β-catenin pathway., Investigational New Drugs. 29 (2011) 273–84. https://doi.org/10.1007/s10637-009-9359-9.

[12] J.Y. Salib, H.N. Michael, E.F. Eskande, Anti-diabetic properties of flavonoid compounds isolated from Hyphaene thebaica epicarp on alloxan induced diabetic rats., Pharmacognosy Research. 5 (2013) 22–9. https://doi.org/10.4103/0974-8490.105644.

[13] T. Boeing, L.M. da Silva, M. Mariott, S.F.D. Andrade, P. de Souza, Diuretic and natriuretic effect of luteolin in normotensive and hypertensive rats: Role of muscarinic acetylcholine receptors, Pharmacological Reports. 69 (2017) 1121–1124. https://doi.org/10.1016/j.pharep.2017.05.010.

[14] G.Y. Lv, Y.P. Zhang, J.L. Gao, J.J. Yu, J. Lei, Z.R. Zhang, B. Li, R.J. Zhan, S.H. Chen, Combined antihypertensive effect of luteolin and buddleoside enriched extracts in spontaneously hypertensive rats, Journal of Ethnopharmacology. 150 (2013) 507–513. https://doi.org/10.1016/j.jep.2013.08.058.

[15] S.F. Nabavi, N. Braidy, O. Gortzi, E. Sobarzo-Sanchez, M. Daglia, K. Skalicka-Woźniak, S.M. Nabavi, Luteolin as an anti-inflammatory and neuroprotective agent: A brief review, Brain Research Bulletin. 119 (2015) 1–11. https://doi.org/10.1016/j.brainresbull.2015.09.002.

[16] N. Aziz, M.Y. Kim, J.Y. Cho, Anti-inflammatory effects of luteolin: A review of in vitro, in vivo, and in silico studies, Journal of Ethnopharmacology. 225 (2018) 342–358. https://doi.org/10.1016/j.jep.2018.05.019.

[17] A.C. Puhl, A. Bernardes, R.L. Silveira, J. Yuan, J.L.O. Campos, D.M. Saidemberg, M.S. Palma, A. Cvoro, S.D. Ayers, P. Webb, P.S. Reinach, M.S. Skaf, I. Polikarpov, Mode of Peroxisome Proliferator-Activated Receptor Activation by Luteolin, Molecular Pharmacology. 81 (2012) 788–799. https://doi.org/10.1124/mol.111.076216.

[18] Q. Qu, J. Qu, Y. Guo, B.-T. Zhou, H.-H. Zhou, Luteolin potentiates the sensitivity of colorectal cancer cell lines to oxaliplatin through the PPARγ/OCTN2 pathway, Anti-Cancer Drugs. 25 (2014) 1016–1027. https://doi.org/10.1097/CAD.0000000000000125.

[19] H.M. El-Bassossy, S.M. Abo-Warda, A. Fahmy, H. El-Bassossy, Chrysin and Luteolin Alleviate Vascular Complications Associated with Insulin Resistance Mainly Through PPAR-° Activation, The American Journal of Chinese Medicine. 42 (2014) 1153–1167. https://doi.org/10.1142/S0192415X14500724.

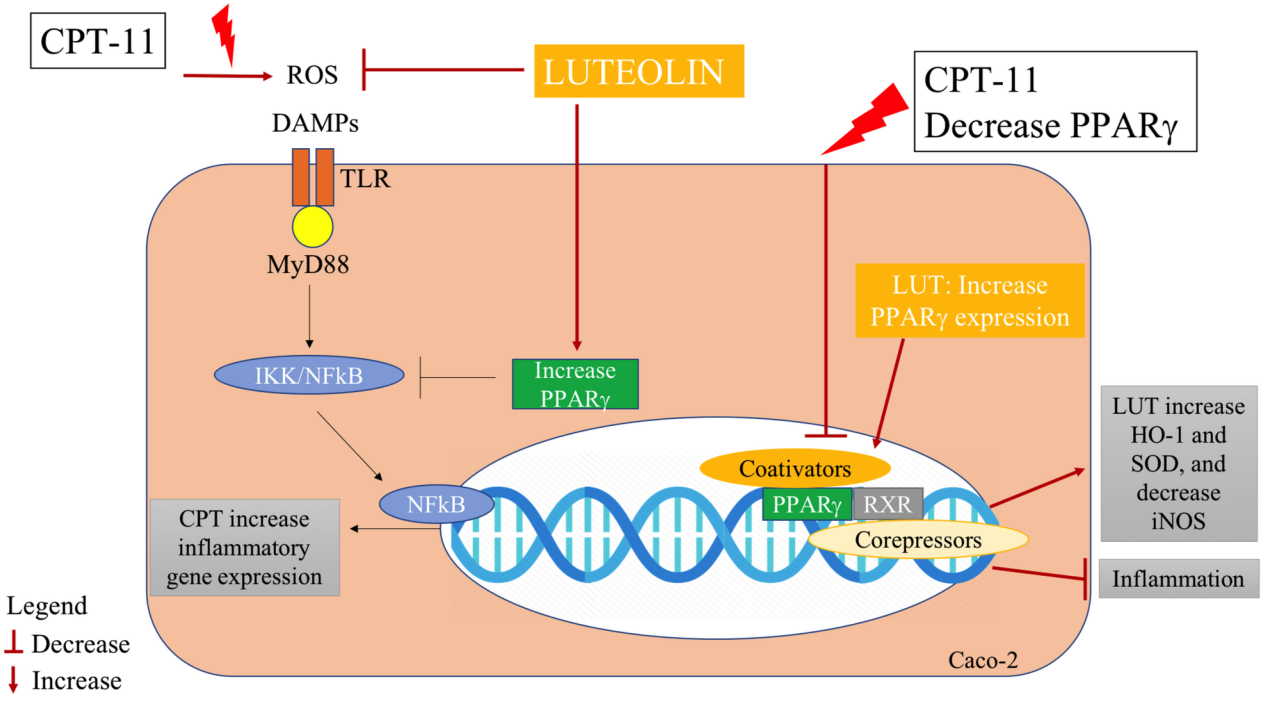
[20] L. Ding, D. Jin, X. Chen, Luteolin enhances insulin sensitivity via activation of PPARγ transcriptional activity in adipocytes, The Journal of Nutritional Biochemistry. 21 (2010) 941–947. https://doi.org/10.1016/j.jnutbio.2009.07.009.

[21] G. Bouguen, A. Langlois, M. Djouina, J. Branche, D. Koriche, E. Dewaeles, A. Mongy, J. Auwerx, J.F. Colombel, P. Desreumaux, L. Dubuquoy, B. Bertin, Intestinal steroidogenesis controls PPARγ expression in the colon and is impaired during ulcerative colitis, Gut. 64 (2015) 901–910. https://doi.org/10.1136/gutjnl-2014-307618.

[22] S. Sauer, Ligands for the Nuclear Peroxisome Proliferator-Activated Receptor Gamma, Trends in Pharmacological Sciences. 36 (2015) 688–704. https://doi.org/10.1016/j.tips.2015.06.010.
[23] C.K. Kontos, M. Avgeris, D. Vassilacopoulou, A. Ardavanis, A. Scorilas, Molecular Effects of Treatment of Human Colorectal Cancer Cells with Natural and Classical Chemotherapeutic Drugs: Alterations in the Expression of Apoptosis-related BCL2 Family Members, Including BCL2L12, Current Pharmaceutical Biotechnology. 19 (2019) 1064–1075. https://doi.org/10.2174/1389201019666181112101410.
[24] D.V.T. Wong, R.C.P. Lima-Júnior, C.B.M. Carvalho, V.F. Borges, C.W.S. Wanderley, A.X.C. Bem, C.A.V.G. Leite, M.A. Teixeira, G.L.P. Batista, R.L. Silva, T.M. Cunha, G.A.C. Brito, P.R.C. Almeida, F.Q. Cunha, R.A. Ribeiro, L. Alexopoulou, The adaptor protein Myd88 is a key signaling molecule in the pathogenesis of irinotecan-induced intestinal mucositis, PLoS ONE. 10 (2015). https://doi.org/10.1371/journal.pone.0139985.
[25] S. Beutheu Youmba, L. Belmonte, L. Galas, N. Boukhettala, C. Bôle-Feysot, P. Déchelotte, M. Coëffier, Methotrexate Modulates Tight Junctions Through NF-κB, MEK, and JNK Pathways, Journal of Pediatric Gastroenterology and Nutrition. 54 (2012) 463–470. https://doi.org/10.1097/MPG.0b013e318247240d.
[26] S. Beutheu, I. Ghouzali, L. Galas, P. Déchelotte, M. Coëffier, Glutamine and arginine improve permeability and tight junction protein expression in methotrexate-treated Caco-2 cells, Clinical Nutrition. 32 (2013) 863–869. https://doi.org/10.1016/j.clnu.2013.01.014.
[27] S.-B. Fang, H.-Y. Shih, C.-H. Huang, L.-T. Li, C.-C. Chen, H.-W. Fang, Live and heat-killed Lactobacillus rhamnosus GG upregulate gene expression of pro-inflammatory cytokines in 5-fluorouracil-pretreated Caco-2 cells, Supportive Care in Cancer. 22 (2014) 1647–1654. https://doi.org/10.1007/s00520-014-2137-z.
[28] J.M. Bowen, A. Tsykin, A.M. Stringer, R.M. Logan, R.J. Gibson, D.M.K. Keefe, Kinetics and regional specificity of irinotecan-induced gene expression in the gastrointestinal tract., Toxicology. 269 (2010) 1–12. https://doi.org/10.1016/j.tox.2009.12.020.
[29] R. Guabiraba, A.G. Besnard, G.B. Menezes, T. Secher, M.S. Jabir, S.S. Amaral, H. Braun, R.C. Lima-Junior, R.A. Ribeiro, F.Q. Cunha, M.M. Teixeira, R. Beyaert, G.J. Graham, F.Y. Liew, IL-33 targeting attenuates intestinal mucositis and enhances effective tumor chemotherapy in mice, Mucosal Immunology. 7 (2014) 1079–1093. https://doi.org/10.1038/mi.2013.124.
[30] R.C.P. Lima, A.A. Figueiredo, H.C. Freitas, M.L.P. Melo, D.V.T. Wong, C.A.V.G. Leite, R.P. Medeiros, R.D. Marques-Neto, M.L. Vale, G.A.C. Brito, R.B. Oriá, M.H.L.P. Souza, F.Q. Cunha, R.A. Ribeiro, Involvement of nitric oxide on the pathogenesis of irinotecan-induced intestinal mucositis: Role of cytokines on inducible nitric oxide synthase activation, Cancer Chemotherapy and Pharmacology. 69 (2012) 931–942. https://doi.org/10.1007/s00280-011-1780-z.
[31] S. Sonis, A Biological Approach to Mucositis, J Support Oncol. 2 (2004) 21–36.
[32] S. Braun, C. Hanselmann, M.G. Gassmann, U. auf dem Keller, C. Born-Berclaz, K. Chan, Y.W. Kan, S. Werner, Nrf2 Transcription Factor, a Novel Target of Keratinocyte Growth Factor Action Which Regulates Gene Expression and Inflammation in the Healing Skin Wound, Molecular and Cellular Biology. 22 (2002) 5492–5505. https://doi.org/10.1128/MCB.22.15.5492-5505.2002.

[33] M. Buelna-Chontal, C. Zazueta, Redox activation of Nrf2 & NF-κB: A double end sword?, Cellular Signalling. 25 (2013) 2548–2557. https://doi.org/10.1016/J.CELLSIG.2013.08.007.
[34] A.Z. Mirza, I.I. Althagafi, H. Shamshad, Role of PPAR receptor in different diseases and their ligands: Physiological importance and clinical implications, European Journal of Medicinal Chemistry. 166 (2019) 502–513. https://doi.org/10.1016/j.ejmech.2019.01.067.
[35] L. Fajas, D. Auboeuf, E. Raspé, K. Schoonjans, A.-M. Lefebvre, R. Saladin, J. Najib, M. Laville, J.-C. Fruchart, S. Deeb, A. Vidal-Puig, J. Flier, M.R. Briggs, B. Staels, H. Vidal, J. Auwerx, The Organization, Promoter Analysis, and Expression of the Human PPARγ Gene, Journal of Biological Chemistry. 272 (1997) 18779–18789. https://doi.org/10.1074/jbc.272.30.18779.
[36] M. Fumery, S. Speca, A. Langlois, A. Davila, C. Dubuquoy, M. Grauso, A. Martin Mena, M. Figeac, D. Metzger, C. Rousseaux, J. Colombel, L. Dubuquoy, P. Desreumaux, B. Bertin, Peroxisome proliferator-activated receptor gamma (PPARγ) regulates lactase expression and activity in the gut, EMBO Molecular Medicine. 9 (2017) e201707795. https://doi.org/10.15252/emmm.201707795.
[37] B. Bertin, L. Dubuquoy, J.-F. Colombel, P. Desreumaux, PPAR-gamma in ulcerative colitis: a novel target for intervention., Current Drug Targets. 14 (2013) 1501–7.
[38] L. Ding, D. Jin, X. Chen, Luteolin enhances insulin sensitivity via activation of PPARγ transcriptional activity in adipocytes., The Journal of Nutritional Biochemistry. 21 (2010) 941–7. https://doi.org/10.1016/j.jnutbio.2009.07.009.
[39] S. Polvani, M. Tarocchi, A. Galli, PPAR and Oxidative Stress: Con() Catenating NRF2 and FOXO, PPAR Research. 2012 (2012) 1–15. https://doi.org/10.1155/2012/641087.
[40] C.K. Tsang, Y. Liu, J. Thomas, Y. Zhang, X.F.S. Zheng, Superoxide dismutase 1 acts as a nuclear transcription factor to regulate oxidative stress resistance, Nature Communications. 5 (2014) 3446. https://doi.org/10.1038/ncomms4446.
[41] H. Was, J. Dulak, A. Jozkowicz, Heme Oxygenase-1 in Tumor Biology and Therapy, (n.d.).
[42] S. Shafi, P. Gupta, G.L. Khatik, J. Gupta, PPARγ: Potential therapeutic target for ailments beyond diabetes and its natural agonism., Current Drug Targets. 20 (2019). https://doi.org/10.2174/1389450120666190527115538.
[43] I. HT, N. JH, TNF alpha and the TNF receptor superfamily: structure-function relationship(s), Microscopy Research and Technique. 50 (2000) 184–195. https://doi.org/10.1002/1097-0029(20000801)50:3<184::aid-jemt2>3.0.co;2-h.
[44] M. M. Fetherolf, S.D. Boyd, D.D. Winkler, D.R. Winge, Oxygen-dependent activation of Cu,Zn-superoxide dismutase-1, Metallomics. 9 (2017) 1047–1059. https://doi.org/10.1039/C6MT00298F.
[45] I.N. Zelko, T.J. Mariani, R.J. Folz, Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression, Free Radical Biology and Medicine. 33 (2002) 337–349. https://doi.org/10.1016/S0891-5849(02)00905-X.
[46] V. Vella, M.L. Nicolosi, S. Giuliano, M. Bellomo, A. Belfiore, R. Malaguarnera, PPAR-γ Agonists As Antineoplastic Agents in Cancers with Dysregulated IGF Axis, Frontiers in Endocrinology. 8 (2017) 31. https://doi.org/10.3389/fendo.2017.00031.

[47] M. Ricote, C.K. Glass, PPARs and molecular mechanisms of transrepression., Biochimica et Biophysica Acta. 1771 (2007) 926–35. https://doi.org/10.1016/j.bbalip.2007.02.013.


CPT-11
ROS
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Decrease PPARγ
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TLR
MyD88
IKK/NFkB
Increase
PPARγ
LUT: Increase
PPARγ expression
Coativators
NFkB
PPARγ
RXR
Corepressors
CPT increase
inflammatory
gene expression
LUT increase
HO-1 and
SOD, and
decrease
iNOS
Inflammation
Caco-2
Legend
Decrease
Increase