Anti-inflammatory and anticancer effects of flavonol glycosides from *Diplotaxis harra* through GSK3β regulation in intestinal cells

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**ABSTRACT**

**Context and objective:** *Diplotaxis harra* (Forssk.) Boiss. (Brassicaceae) is traditionally used as an antidiabetic, anti-inflammatory or anticancer agent. In these pathologies, the glycogen synthase kinase 3β (GSK3β) is overactivated and represents an interesting therapeutic target. Several flavonoids can inhibit GSK3β and the purpose of this study was to search for the compounds in *Diplotaxis harra* which are able to modulate GSK3β.

**Materials and methods:** Methanol extracts from *D. harra* flowers were prepared and the bio-guided fractionation of their active components was performed using inflammatory [protease-activated receptor 2 (PAR2)-stimulated IEC6 cells] and cancer (human Caco-2 cell line) intestinal cells. 50–100 µg/mL of fractions or compounds purified by HPLC were incubated with cells whose inhibited form of GSK3β (Pser9 GSK3β) and survival were analyzed by Western blot at 1 h and colorimetric assay at 24 h, respectively. LC-UV-MS profiles and MS-MS spectra were used for the characterization of extracts and flavonoids-enriched fractions, and the identification of pure flavonoids was achieved by MS and NMR analysis.

**Results:** The methanol extract from *D. harra* flowers and its flavonoid-enriched fraction inhibit GSK3β in PAR2-stimulated IEC6 cells. GSK3β inhibition by the flavonoid-enriched *D. harra* fraction was dependent on PKC activation. The flavonoid-enriched *D. harra* fraction and its purified compound isorhamnetin-3,7-di-O-glucoside induced a 20% decrease of PAR2-stimulated IEC6 and Caco-2 cell survival. Importantly, normal cells (non-stimulated IEC6 cells) were spared by these treatments.

**Conclusion:** This work indicates that flavonoids from *D. harra* display cytotoxic activity against inflammatory and cancer intestinal cells which could depend on GSK3β inhibition.

**Introduction**

Inflammatory bowel diseases (IBD) are frequent pathologies that result from the interaction between genetic factors and microbial and environmental cues. The patients with long-standing IBD, such as ulcerative colitis and Crohn’s disease, have an increased risk of developing colorectal cancer (CRC) (Triantafillidis et al. 2009). Even though anti-inflammatory therapies and colonoscopy surveillance have decreased the incidence of colitis-induced CRC, new therapeutic approaches are needed to avoid therapeutic resistance and complications.

Diet has been implicated in the development and therapy of IBD (Neuman & Nanau 2012). For example, diet low in fruits and vegetables leads to an increased risk of developing IBD and CRC (Pan et al. 2011). Important phytochemicals of the human diet such as flavonoids and isothiocyanates have chemoprotective effects in a number of animal models of experimental IBD and CRC (Pan et al. 2011; Dinkova-Kostova 2012). Dietary consumption of flavonoids has been inversely associated with the risk of CRC (Rossi et al. 2010).

Intestinal stem cells (ISC) homeostasis is impaired in IBD and CRC (Vermeulen & Snippert 2014). The serine-threonine kinase, glycogen synthase kinase 3 β (GSK3β), plays a critical role in the regulation of important ISC functions such as Wingless-related integration site (Wnt) proliferative response, NFκB and telomerase survival pathways, and Hath1 differentiation activity (Steinbrecher et al. 2005; Tsuchiya et al. 2007; Mai et al. 2009).

Also, GSK3β influences barrier functions through the regulation of adherens junctions and Rho kinase (Severson et al. 2010). In IBD and CRC, GSK3β is overexpressed/overactivated and its inhibition ameliorates colitis and CRC response to chemotherapy (Whittle et al. 2006; Wang et al. 2011; Grassilli et al. 2013). Interestingly, we recently demonstrated that both in inflammatory and cancer intestinal models, i.e., protease-activated receptor 2 (PAR2)-stimulated colonospheres or IEC6 cells and Caco-2 spheroids, a PAR2-GSK3β pathway controls primitive cell survival and proliferation (Nasri et al. 2016).

We have previously shown that it is possible to specifically target pathological stem cells through the modulation of GSK3β by glucosflavonoids. Indeed, in leukemic stem cells, flavonoids whose structure is close to rutin (quercetol-3-O-glucose-rhamnose) induce apoptosis through the enhancement of the Akt/GSK3β pathway (Bourogaa et al. 2011). Interestingly, dietary rutin ameliorates experimental colitis and colon carcinogenesis through the attenuation of pro-inflammatory gene expression
and of biotransforming and bacterial enzymes activities (Kwon et al. 2005; Vinothkumar et al. 2014).

It has been shown that a mixture of flavonoids prevents intestinal polyps and reduces the recurrence of CRC after resection (Hoensch et al. 2008; Fini et al. 2011). Looking for compounds that could synergize with rutin in inflammation and cancer therapies, we have investigated the phytochemical composition of Diplotaxis harra (Forsk.) Bois. (Brassicaceae). Indeed, D. harra is an edible plant used in traditional medicine for the treatment of various diseases such as anemia, hypercholesterolemia, diabetes and mainly inflammation and cancer (Bellakhdar 1997; Ghrabi 2005; Ramadan et al. 2009). The aerial parts of this medicinal plant have a high polyphenol content that could support anti-inflammatory and anticancer properties (Falleh et al. 2013; Kassem et al. 2013). Our purpose was to determine if compounds from D. harra could modulate epithelial GSK3β activation in two in vitro models: PAR2-stimulated IEC6 cells, mimicking inflammatory conditions (Li et al. 2015), and human colon cancer-derived Caco-2 cell line grown in 3D.

**Materials and methods**

**Plant material**

*Diplotaxis harra* (leaves, flowers and roots) was collected from Sidi Bouzid, in the center of Tunisia, in December 2011 and authenticated by Prof. Mohamed Chaieb, Biology Department, University of Sfax. A voucher specimen (LCSN130) was deposited in the Herbarium of the Laboratory of Chemistry of Natural Substances, Department of Chemistry, Faculty of Sciences of Sfax, Tunisia.

**Extraction and isolation**

The different parts of *D. harra* [leaves (800 g), flowers (220 g) and roots (2.4 kg)] were air-dried in shade, defatted with *n*-hexane and then macerated thrice during 24 h successively with ethyl acetate and methanol. For each extraction, the used solvent volumes were 1 L for leaves and flowers and 3 L for roots. After filtration, the solvent was evaporated using a rotary evaporator (Heidolph, Germany). Close examination of the extracts separated by thin layer chromatography (TLC, silica-gel 60 Å, 40–63 µm) developed with ethyl acetate:butanol-water:acetic acid (5:4:0.5:0.5) solvent mixture and observed under UV light at 254 and 366 nm with the presence of ammonia vapour revealed the presence of spots of flavonoids in methanol extracts. Flower methanol extract (6 g) was separated by column chromatography on silica gel (Kieselgel 60 Å (40-63 µm, PF254) and eluted with mixtures of ethyl acetate:methanol of increasing polarity (100:0 — 0:100) to yield 9 fractions pooled according to their TLC profiles (same TLC parameters as described above). LC-MS/MS analysis (see below for details) of all the fractions showed the presence of flavonoids in fractions 3 (F3) and 6 (F6). Fraction F6 (2 g) was chromatographed over a C18 silica gel column (RP-18 Silica 60 Å, 40–60 µm) and eluted gradually with water:acetonitrile (100:0 — 0:100), yielding isorhamnetin-3-O-glucoside (31 mg) and 13 sub-fractions (L1-L13). MS/MS analysis of all sub-fractions showed the presence of flavonoids in two sub-fractions (L7 and L12).

L12 (100 mg) was dissolved in 10 mL of methanol and then 1 mL of this solution was injected in preparative HPLC (see below for details). The major product of this fraction, isorhamnetin-3,7-di-O-glucoside (23 mg), was eluted isocratically at 3.89 min in a volume of 0.1% formic acid:acetonitrile (80:20). Purity of the isolated compound was 95% determined by UPLC/DAD analysis.

Identification of isorhamnetin mono- and di-glucosides was carried out by MS and 1D and 2D NMR analysis and the data compared with literature (Igarashi et al. 2008; Liu et al. 2010).

**LC-MS/MS analysis**

The identification of flavonoids in the extracts was performed by LC-MS using an ULTIMATE 3000 HPLC system coupled to an ion trap mass spectrometer (LCQ DECA XP Max, Thermo Finnigan) equipped with an electrospray source functioning in the negative ion mode. Extracts were separated on a Phenomenex column (Kinetex C18, 150 × 4.6 mm, 5 µm) with a gradient from 90% (+0.1% formic acid) to 100% acetonitrile.

**Preparative HPLC**

Semi-preparative HPLC was performed on an auto purification system equipped with a Photodiode Array Detector (Waters). The column was a Waters X bridge C18 5 µm (150 × 19 mm). 0.1% formic acid and 100% acetonitrile were respectively employed as solvents A and B with a flow rate of 20.5 mL/min. The elution gradient was 6.5 min gradient run from 5% to 95% B in 6.5 min and then returned to initial conditions. The elution was monitored by UV detection at 254 and 355 nm. The collected fractions were pooled and analyzed by analytical UPLC on a UPLC Acquity system from Waters with an Acquity BEH C18 1.7 µm (2.1 × 50 mm) column at 40 °C. The isocratic conditions were water:acetonitrile (80:20). The flow rate was 0.6 mL/min and the detection was set at 254 nm.

**Cell culture**

IEC6 (ATCC-CRL-1592) cells from rat small intestine and Caco-2 (ATCC-HTB-37; LGC Standards authentication certificate) cells from human CRC were purchased from ATCC (LGC Standards, Molsheim, France) and were cultured in Dulbecco’s modified Eagle’s medium (DMEM Cat. No. 31966 with Glutamax and 1 mM sodium pyruvate) supplemented with 100 U/mL penicillin/streptomycin and 10% fetal calf serum (FCS) without complement at 37 °C and 5% CO2. Caco-2 culture medium was supplemented with 1% non-essential amino acids. All cell culture reagents were from Invitrogen (Carlsbad, CA). Once the cells reached 90% confluency, cells were used for experiments.

In 2D culture, IEC6 and Caco-2 cells were seeded in 12-well plates (0.5 × 10⁶ cells) or 96-well plates (4 × 10⁵ cells) and were incubated with DMEM and 10% FCS at 37 °C for 24 h. In 3D Culture, 1 × 10⁵ Caco-2 cells were embedded in 4 µL Matrigel seeded on top of 20 µL polymerized Matrigel in 48-well plates. Cells were cultured in DMEM and 10% FCS and 1% non-essential amino acids at 37 °C for 24 h. In these conditions, cells were growing as spheroids that we observed daily using an aperture microscope (Zeiss Axio-observer, HXP120).

After 24 h of 2D or 3D culture, cells were serum-starved for 24 h before stimulation by 100 µM PAR2 agonist peptide (PAR2 AP, Genscript, Piscataway, NJ) or its inverted control (Ezbiolab Inc., Carmel, IN) for 1 h. Methanol extracts of *D. harra* organs and fractions F3 and F6 (solubilized in ethanol 10%, 10 mg/mL), and sub-fraction L12 and isorhamnetin-3-O-glucoside and isorhamnetin-3,7-di-O-glucoside (dissolved in DMSO 50%,...
10 mg/mL) were then incubated with cells at 100 or 150 µg/mL (fractions) or 10-50 µg/mL (purified compounds). The protein kinase C (PKC) inhibitor Go6976 (0.2 µM, Sigma-Aldrich, Saint-Quentin Fallavier, France) was added 15 min before F6. In some experiments, GSK3 pharmacological inhibitors (SB216763 12.5 µM, BIO 500 nM or CHIR99021 2.5 µM, Tocris Bioscience, RD Systems, Lille, France) were incubated with cells in 3D culture. DMSO was used as solvent for pharmacological inhibitors and used at a maximal 0.05% final concentration. After 1 h of incubation, 12-well plates were processed to prepare cell lysates used for Western blot and after 24 h, 96-well and 48-well plates were incubated with MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) to measure surviving cells.

**Western blot analysis**

Cellular proteins were extracted from IEC6 and Caco-2 cells and subjected to Western blotting analysis (Nasri et al. 2016), to examine the regulation of GSK3β using primary antibodies Pser9GSK3β (diluted 1:1000) and Pser473Akt (diluted 1:1000) from Cell Signalling Technology (Ozyme, Saint Quentin Yvelines, France), GSK3β (diluted 1:2500) from BD Biosciences (Le Pont de Claix, France), Ptyr307PP2A (diluted 1:1000) and actin (diluted 1:1000) from Abcam (Paris, France). Signals were detected using a chemiluminescent substrate (SuperSignal, Amersham Pharmacia Biotech, Piscataway, NJ).

**Cell survival/proliferation assay**

Cells were incubated with MTS for 3 h, at 20 µL/well (96-well plates) or 40 µL/well (48-well plates). Then, absorbance was measured at 480 nm by spectrometry (Varioskan Flash, Thermo Fisher Scientific, Illkirch, France). The percentage of surviving cells was determined by comparing the absorbances of assays and controls (non-treated cells). The test was carried out by triplicate in 2D culture and by duplicate in 3D culture.

**Statistical analysis**

Student’s t-test was used for the experiment’s analysis.

**Results**

**Diplotaxis harra flower methanol extract modulates GSK3β**

In search of flavonoids in *D. harra* able to modulate GSK3β, we performed methanol extraction of flowers, roots and leaves. Extracts were then tested on the PAR2 AP-stimulated IEC6 cell model. As shown in Figure 1(A), inhibited β isoform of GSK3 (Pser9 GSK3β) was specifically increased compared to controls (non-treated cells). The test was carried out by triplicate in 2D culture and by duplicate in 3D culture.

**Flavonoid-enriched fraction from Diplotaxis harra flowers modulates GSK3β through PKC**

We had previously shown that the phosphatase PP2A was implicated in the PAR2-dependent activation of GSK3β in IEC6 cells and that the modulatory kinase of GSK3β, Akt, was concomitantly inhibited (Nasri et al. 2016). We thus investigated whether the flavonoid-enriched fraction F6 may regulate PP2A and Akt. As shown in Figure 2(A), treatment with F6 fraction did not affect the regulation of PP2A and Akt.

**Figure 1.** Flavonoids from *D. harra* flowers modulate GSK3β. (A) Serum-starved IEC6 cells were stimulated with PAR2 AP (100 µM) for 1 h and then incubated for 1 h with methanol extracts from *D. harra* leaves, flowers or roots (100 µg/mL) or ethanol/water (10%) as control. The variations of the inhibited form of GSK3β (Pser9 GSK3β) were analyzed with total protein GSK3β by Western blot. Representative of three independent experiments. (B) Flavonoid-enriched fractions of *D. harra* flowers, F6 and F3 (100 µg/mL), were incubated with IEC6 cells as described in (A). Variations of Pser9 GSK3β were analyzed after this treatment in IEC6 cells previously stimulated with PAR2 AP or control peptide (100 µM). Actin is shown as loading control. Representative of two independent experiments.

**Figure 2.** The flavonoid-dependent modulation of GSK3β is regulated by PKC. Serum-starved IEC6 cells were stimulated with PAR2 AP (100 µM) for 1 h and then incubated for 1 h with the flavonoid-enriched fraction F6 from *D. harra* flowers (100 µg/mL) or ethanol/water (10%) as control. In A, Ptyr307PP2A (inhibited form) and Pser473Akt (activated form) were analyzed by Western blot. Representative of two independent experiments. In B, PAR2 AP-stimulated IEC6 cells were treated with 0.2 µM PKC inhibitor Go6776 or DMSO (solvent) for 15 min before F6 addition. Pser9 GSK3β and total GSK3β were analyzed by Western blot. Representative of two independent experiments.
change P307PP2A (inhibited form) and P473Akt (activated form) in PAR₂-stimulated IEC6 cells, demonstrating that F6 does not modulate GSK3β through PP2A and Akt.

Conversely, inhibition of PKC, another modulatory kinase of GSK3β, prevented serine 9 phosphorylation of GSK3β induced by F6 treatment in PAR₂-stimulated IEC6 cells (Figure 2(B)). Thus, F6 inhibits GSK3β through PKC in PAR₂-stimulated IEC6 cells.

These results suggest that flavonoids from D. harra flowers might modulate GSK3β through PKC regulation.

**Flavonoid-enriched fraction from Diplotaxis harra flowers is cytotoxic for inflammatory and cancer intestinal cells**

Given the implication of GSK3β in intestinal inflammation and cancer, we have then investigated the impact of the flavonoid-enriched fraction F6 on cell survival in PAR₂-stimulated intestinal IEC6 cells and colon cancer Caco-2 cells. As shown in Figure 3(A), the percentage of surviving IEC6 cells decreased after 48h of serum starvation. However, PAR₂ AP treatment prevented partially this decrease and we have shown previously that the expression of active caspase 3 was concomitantly down-regulated suggesting a protective role of PAR₂ against serum starvation-induced apoptosis in IEC6 cells (Nasri et al. 2016). Interestingly, F6 treatment increased IEC6 cells survival in serum-starved conditions but decreased it upon pretreatment by PAR₂ AP (Figure 3(A)). Pharmacological GSK3 inhibition has been shown to induce cytotoxicity in colon cancer cells (Shakoori et al. 2007) and indeed Caco-2 cells cultured in 3D were strongly decreased after GSK3 inhibition using either Chir99021, BIO or SB216763 treatment (Figure 3(A)). F6 treatment also induced a 20% decrease in Caco-2 cells both in 2D and 3D culture conditions (Figure 3(A)). These results are in agreement with the pro-survival role of GSK3β in inflammation and cancer and strongly suggest that the flavonoid-enriched fraction of D. harra flowers is cytotoxic for inflammatory and cancer intestinal cells through GSK3β modulation.

We then analyzed F6 composition by LC-MS/MS in search of glucosylflavonoids that we previously found active to target pathological cells through the modulation of GSK3β. As shown in Figure 3(B), examination of the negative ion Total Ion Current chromatogram of the F6 fraction. (C) MS/MS spectra of main ions detected in F6.

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(TIC) chromatogram of the flavonoid-enriched fraction F6 showed the presence of two major groups of glucosflavonoids. Close examination of MS-MS spectra (Figure 3(C)) of four main ions detected in F6 led us to propose the presence of mono- and di-heterosides possessing isorhamnetin (ion at $m/z$ 315), kaempferol (ion at $m/z$ 285) and quercetin (ion at $m/z$ 301) as genins, all already described in *D. harra* (Atta et al. 2011; Kassem et al. 2013).

These results suggest that one or several glucosflavonoids present in F6 could trigger cytotoxicity in pathological intestinal cells through GSK3β inhibition.

**Isorhamnetin-3,7-di-O-glucoside purified from Diplotaxis harra flowers has anti-inflammatory and anticancer properties in vitro**

Further purification of compounds in fraction F6 was realized using column chromatography (water-acetonitrile gradient). As shown in Figure 4(A), one of sub-fractions obtained, L12, displayed interesting cytotoxicity, decreasing strongly cancer Caco-2 cells while sparing non-transformed IEC6 cells. The full MS spectrum [ESI(-)] of L12 (Figure 4(B)) displayed 2 main ions at $m/z$ 639 and 477. Close examination of MS-MS spectra of these two main ions led us to propose the presence of isorhamnetin-glucosides since they led to the same product ion at $m/z$ 315 ([M-H]⁻ of isorhamnetin) (Figure 4(B)).

Purification process using C18 reversed-phase silica gel HPLC led to the isolation of isorhamnetin-3,7-di-O-glucoside (ion at $m/z$ 639 [M-H]⁻) and isorhamnetin-3-O-glucoside (ion at $m/z$ 477 [M-H]⁻), that were identified according to their MS and NMR spectral data and by comparison with those of literature. Both compounds have already been described in the whole plant *D. harra* (Kassem et al. 2013). Interestingly, isorhamnetin-3,7-di-O-glucoside, but not isorhamnetin-3-O-glucoside (not shown), displayed cytotoxicity in PAR2-stimulated IEC6 cells and Caco-2 cells (Figure 5).

Thus, these results show that isorhamnetin-3,7-di-O-glucoside may be involved in anti-inflammatory and anticancer properties of *D. harra* flowers.

**Discussion and conclusions**

The main finding of this study elucidated the anti-inflammatory and anticancer effects of the medicinal plant *Diplotaxis harra* that could be partially supported by flavonol glycosides through GSK3β regulation.

We found that a flavonoid-enriched fraction of *D. harra* flowers modulates GSK3β. Previous studies have predicted that some
flavonoids could fit into the binding pocket of GSK3β (Johnson et al. 2011). However, we show that in intestinal cells, PKC is implicated in the GSK3β inhibition by flavonoids. Indeed, Go6976, an inhibitor of PKCα and PKCβ, impaired serine 9 phosphorylation (inhibition) of GSK3β triggered by the flavonoid-enriched fraction of D. harra flowers. Interestingly, such PKC-dependent inhibition of GSK3β has already been described in human colon cancer cells and correlated with increased cell proliferation (Wang et al. 2006). Thus, in our experimental conditions, the cytotoxic effect of flavonoids on inflammatory and cancer intestinal cells may be related to an exit from cell quiescence.

Also, PKC has been shown to play an important role in the cytoskeleton organization of intestinal cells (Fasano et al. 1995) and we have shown that the Rho kinase controls GSK3β activity in PAR2-stimulated intestinal cells (Nasri et al. 2016). Therefore, it is possible that flavonoids, through an impact on the cytoskeleton (Noda et al. 2013) and consequently on PKC activity, down-regulate GSK3β. Importantly, treatment with the flavonoid-enriched fraction of D. harra flowers was not deleterious for non-inflammatory or non-transformed cells.

LC-MS/MS analysis of the flavonoid-enriched fraction F6 of D. harra indicated the presence of seven flavonol glycosides displaying three kinds of aglycones (isorhamnetin, quercetin and kaempferol). According to their MS profiles, these heterosides were putatively identified as isorhamnetin-3-O-glucoside (ion at m/z 477 [M-H]-); isorhamnetin-3,7-di-O-glucoside (ion at m/z 639 [M-H]-); quercetin-3,4-di-O-glucoside (ion at m/z 625 [M-H]-); kaempferol-3,4-di-O-glucoside (ion at m/z 609 [M-H]-); kaempferol-3-di-O-glucoside (ion at m/z 609 [M-H]-); isorhamnetin-3,4'-di-O-glucoside (ion at m/z 639 [M-H]-) and isorhamnetin-3,4'-di-O-glucoside (ion at m/z 639 [M]-) (Figure 6).

Given our previous work showing that rutin is cytotoxic for leukemic progenitors (Bourogaa et al. 2011), these flavonol glycosides could be responsible for the GSK3β-dependent cytotoxicity in pathological intestinal cells. This is in accordance with the results of Mohammed et al. (2013) showing cytotoxicity of isolated flavonol glycosides from D. harra (quercetin-3-O-β-glucoside, isorhamnetin-7-O-β-glucoside, apigenin-7-O-β-rhamnoside and kaempferol-3-O-β-glucoside) against the human colon cancer HCT116 cell line. However, for the first time, we show that the compound isorhamnetin-3,7-di-O-glucoside purified from
D. harra flowers, has cytotoxic activities against cells displaying GSK3β activation, namely here as an example cancer intestinal cells and intestinal cells under inflammatory signal conditions. As with the flavonoid-enriched extract from D. harra flowers, non-pathological cells were spared by this treatment.

We have previously shown (Bourogaa et al. 2011) that the GSK3β-based cytotoxicity of flavonol glycosides requires either the association of glucose-rhamnose (= rutinoside) [rutin (= quercetin-3-O-rutinoside), isorhamnetin-3-rutinoside], or rhamnose (quercitrin), or galactose (hyperoside) in the position 3 of a flavonol structure. Indeed, in contrast to isorhamnetin-3,7-di-O-glucoside, we found that the second flavonol glycoside purified from D. harra flowers, isorhamnetin-3-O-glucoside, was not cytotoxic for inflammatory and cancer intestinal cells. Moreover, in our previous data (Bourogaa et al. 2011), robinin (kaempferol-3-O-galactose-rhamnose-7-O-rhamnose), but not quercetin-7-O-glucoside, displayed cytotoxic activity as rutin, suggesting that the position 7 of flavonol structure is also of interest when associated with glycosylation in position 3. These results are in agreement with Antunes-Ricardo et al. (2014) who showed that glycosylation affects the anti-proliferative effect of the extract of Opuntia ficus-indica (L.) Mill. var Jalpa (Cactaceae) and that the isorhamnetin diglycosides were more cytotoxic than pure isorhamnetin aglycone against colon cancer HT-29 cells.

In conclusion, this study shows that GSK3β can be targeted by natural compounds such as flavonol glycosides in inflammatory and cancer intestinal pathologies, and that isorhamnetin-3,7-di-O-glucoside is a new member of this GSK3β-targeting flavonoid family.

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