

## Quercetin-3-O-glucoside Induces Human DNA Topoisomerase II Inhibition, Cell Cycle Arrest and Apoptosis in Hepatocellular Carcinoma Cells

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**Abstract.** *Background: Dietary flavonoids have been associated with reduced risk of cancer including hepatocellular carcinoma (HCC). Quercetin-3-O-glucoside (Q3G) has been shown to possess anti-proliferative and antioxidant activities. The objectives of this study were to assess the anti-proliferative properties of Q3G in human liver cancer cells (HepG2); assess the cytotoxicity on normal primary cells; and elucidate its possible mechanism of action(s). Materials and Methods: Using a dose- and time-dependent study, we evaluated the antiproliferative properties of Q3G in HepG2 cells using MTS cell viability assay and lactate dehydrogenase release assay. To elucidate the mechanism of action, we performed cell-cycle analysis using flow cytometry. Cell death via apoptosis was analyzed by DNA fragmentation assay, caspase-3 induction assay and fluorescence microscopy. DNA topoisomerase II drug screening assay was performed to assess the effect of Q3G on DNA topoisomerase II. Results: Q3G treatment inhibited cell proliferation in a dose- and time-dependent manner in HepG2 cells with the blockade of the cell cycle in the S-phase. Additionally, Q3G exhibited a strong ability*

*to inhibit DNA topoisomerase II. Furthermore, DNA fragmentation and fluorescence microscopy analysis suggested that Q3G induced apoptosis in HepG2 cells with the activation of caspase-3. Interestingly, Q3G exhibited significantly lower toxicity to normal cells (primary human and rat hepatocytes and primary lung cells) than sorafenib ( $p < 0.05$ ), a chemotherapy drug for hepatocellular carcinoma. The results suggest that Q3G is a potential antitumor agent against liver cancer with a possible mechanism of action via cell-cycle arrest and apoptosis. Further research should be performed to confirm these results in vivo.*

HCC is the third ranked cause of death by cancer worldwide (1). Current treatments for hepatocellular carcinoma (HCC) or liver cancer include surgery and chemotherapy; however, use of chemotherapy drugs such as sorafenib is not efficient due to numerous side-effects. Therefore, searching for and developing safe and efficient anti-tumor drugs for liver cancer remains a demand in therapeutic research.

Increased consumption of fruits and vegetables which are rich in polyphenols such as flavonoids has been associated with a reduced risk of cancer (2, 3). Quercetin is one of the most common flavonoids found in the diet (3). Its anti-proliferative properties have been reported by various studies (4, 5). Quercetin-induced cell death and reduction in cancer cell proliferation has been primarily associated with apoptotic mechanisms in various cancer cell lines including the HepG2 human liver cancer cell line (6-11). Additionally, apoptosis induction by quercetin has also been associated with regulation of cell cycle and various signaling molecules including caspase-3 (12-15).

Flavonoids in their natural form exist as glycosides with different sugar moieties (16). Quercetin-3-O-glucoside (Q3G) is a natural-occurring form of quercetin and has been shown to exhibit strong antioxidant activity (17). Recent studies showed potential anti-proliferative activity of

**Abbreviations:** ATCC: American Type Culture Collection; DEVD: pNA- Asp-Glu-Val-Asp p-nitroanilide; DMSO: dimethyl sulphoxide; EDTA: ethylenediaminetetraacetic acid; LDH: lactate dehydrogenase; MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NHEPS: human hepatocyte cells; PI: propidium iodide; Q3G: Quercetin-3-O-glucoside; RTCP-10: rat (Sprague-Dawley) cryopreserved hepatocytes, plateable male; WI-38: human lung fibroblasts.

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**Key Words:** Quercetin-3-O-glucoside, cancer, apoptosis, cell cycle, caspase-3, topoisomerase II, HepG2 cells.

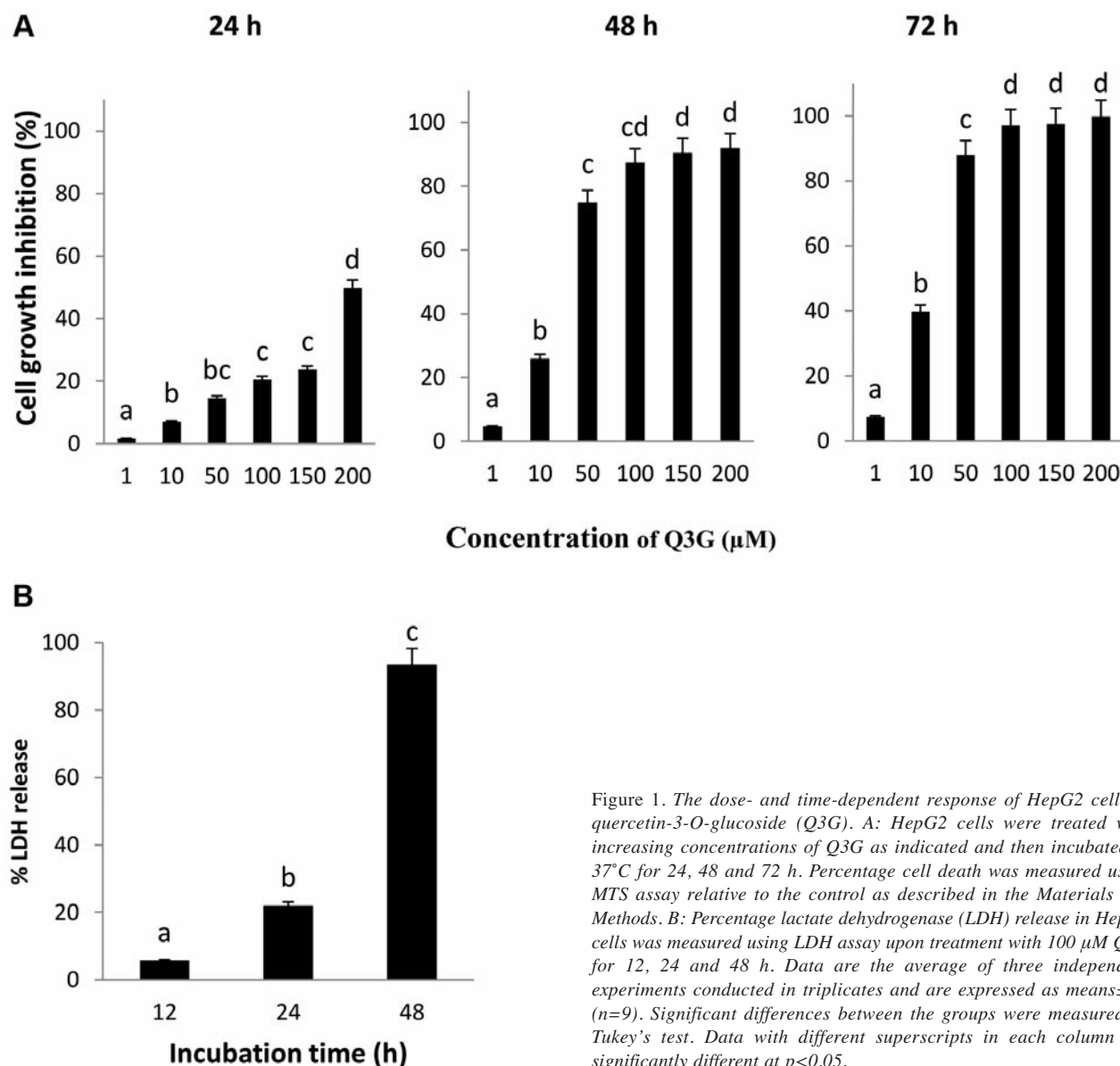


Figure 1. The dose- and time-dependent response of HepG2 cells to quercetin-3-O-glucoside (Q3G). A: HepG2 cells were treated with increasing concentrations of Q3G as indicated and then incubated at 37°C for 24, 48 and 72 h. Percentage cell death was measured using MTS assay relative to the control as described in the Materials and Methods. B: Percentage lactate dehydrogenase (LDH) release in HepG2 cells was measured using LDH assay upon treatment with 100  $\mu\text{M}$  Q3G for 12, 24 and 48 h. Data are the average of three independent experiments conducted in triplicates and are expressed as means $\pm$ SD (n=9). Significant differences between the groups were measured by Tukey's test. Data with different superscripts in each column are significantly different at  $p<0.05$ .

quercetin and its glycosides including Q3G (18). Furthermore, synergistic effect of apple extracts and Q3G has been shown to enhance the antiproliferative activity in human breast cancer MCF-7 cells (19). However, a time-dependent study of Q3G antitumor activity and mechanism of action in HepG2 cells has not been yet reported.

In the present study, we performed a time- and dose-dependent study and characterized the anti-tumor activity and possible mechanism of action of Q3G in HepG2 cells. Additionally, this study also involved comparative analysis with sorafenib (Nexovar®), a currently prescribed chemotherapy drug for liver cancer.

## Materials and Methods

**Materials and chemicals.** Propidium iodide, Dimethyl sulfoxide (DMSO) and two-well chambered cover slides were purchased from Sigma-Aldrich (Mississauga, ON, Canada). Sorafenib (Nexovar®) was purchased from Cayman Chemical (Ann Arbor, Michigan). All cell culture vessels and plates were purchased from BD Biosciences (San Jose, CA, USA). The BCA protein purification kit was purchased from Thermo Scientific (Burlington, ON, Canada).

**Cell culture and reagents.** HepG2 cells (ATCC#8065) and WI-38 lung cells (ATCC#CCL-75™) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and

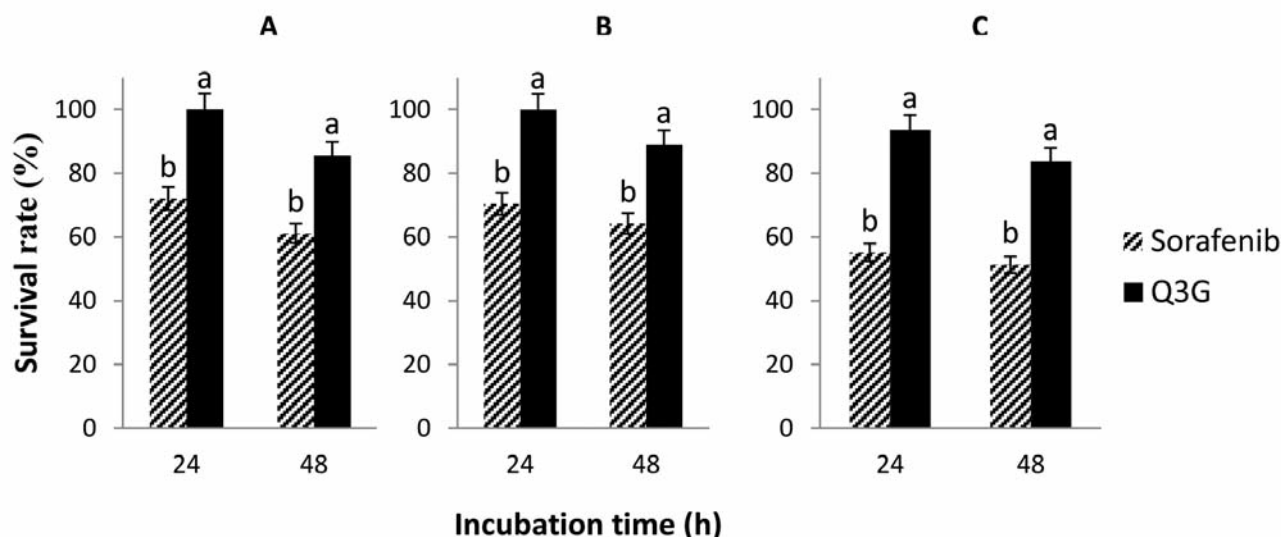


Figure 2. Effect of quercetin-3-O-glucoside (Q3G) on cell viability of three primary cell lines: primary human hepatocytes (NHEPS), primary rat hepatocytes (RTCP-10) and primary human lung cells (WI-38). The figure depicts the percentage of viable primary NHEPS (A), RTCP-10 (B) and WI-38 (C) cells after treatment with Q3G and sorafenib. Cells ( $1 \times 10^4$  cells per well; 96-well plate) were treated with 100  $\mu$ M Q3G or sorafenib for 24 and 48 h. After treatment, the viable cell percentage was determined by MTS assay as described in Materials and Methods. Results are expressed relative to those of the control (24 and 48 h incubation without test compounds). Mean separation between groups was conducted using the Tukey's test ( $n=6$ ). Data with different superscripts in each column are significantly different. Significance level was set at  $p < 0.05$ .

maintained according to their instructions, in using standard growth conditions (37°C, 5% CO<sub>2</sub> and 90% humidity) in T-75 tissue culture flasks. Sub-culturing was performed every two to three days when cells reached a confluency of 70-80%. Primary human hepatocytes (NHEPS™) were purchased from Lonza (Mississauga, ON, Canada) and primary rat hepatocytes (RTCP-10) were purchased from Invitrogen (Burlington, ON, Canada) and were cultured according to the supplier's instructions. Cells were counted under Nikon Eclipse TS 100 phase-contrast microscope (Mississauga, ON, Canada) using a hemocytometer and then transferred to fresh flasks.

**Measurement of cell viability.** The assay was performed using Cell Titer 96™ Aqueous One solution cell proliferation (MTS) assay kit (Promega Madison, WI, USA) according to the manufacturer's instructions. Briefly, cells were seeded in a 96-well microplate at a density of  $2 \times 10^4$  cells per well. The microplates were placed in a culture incubator under standard conditions and cultured for 24 h. After incubation, the cells were treated with six different doses (1, 10, 50, 100, 150 and 200  $\mu$ M) of Q3G in fresh media in triplicates. The concentration of DMSO used to prepare test solutions in all the assays was less than 1%. The plates were then incubated for different time intervals (24, 48 and 72 h) under standard growth conditions. According to the kit instructions, MTS reagent was added to each well (5 g/l in phosphate buffer saline (PBS)) and cells were again incubated for 2 h. The absorbance of each well was recorded directly at 490 nm using FluoStar Optima microplate reader (BMG Labtech, Ortenberg, Germany).

**Measurement of cell cytotoxicity.** The assay was performed using CytoTox 96® Non-Radioactive Cytotoxicity Assay kit (Promega),

according to the manufacturer's instructions. Briefly, HepG2 cells (5,000 cells/well) were plated in 96-well microplates. For the positive control, cells were treated with Triton X-100 to achieve maximum lactate dehydrogenase (LDH) release. The microplates were kept in a culture incubator under standard conditions and cultured for 24 h. After incubation, Q3G at a concentration of 100  $\mu$ M in fresh media was added to each well in triplicates. The plates were incubated for different time intervals (6, 12, 18 and 24 h) under standard growth conditions. After treatment, the 96-well microplate was centrifuged and supernatant was transferred to a fresh 96-well microplate and subjected to LDH assay. Absorbance was measured at 490 nm using FluoStar Optima microplate reader (BMG Labtech, Ortenberg, Germany).

**DNA fragmentation.** The assay was performed using ApoTarget™ Quick Apoptotic DNA Ladder Detection Kit (Invitrogen, Burlington, ON, Canada) according to the manufacturer's instructions. Briefly, HepG2 cells ( $5 \times 10^5$  cells/well) were grown in a 12-well culture plate (75-80%) confluency and then treated with 100  $\mu$ M Q3G for 24, 48 and 72 h. Cells were collected and total DNA was isolated from each sample. Extracted DNA pellet was dissolved in 30  $\mu$ l of DNA suspension buffer (provided with the kit) and resolved on 1.2% agarose gel containing GelRed™ stain in 1X TAE buffer (pH 8.5, 20 mM Tris-acetic acid, 2 mM EDTA) in BioRad mini-gel electrophoresis kit (Mississauga, ON, Canada). The DNA gel was visualized and photographed by BioRad's Gel Doc™ EZ system.

**Measurement of caspase-3 activity.** The assay was performed using Caspase-3/cysteine protease protein-32 (CPP32) Colorimetric Assay Kit (BioVision, Inc. Milpitas, CA, USA) according to the

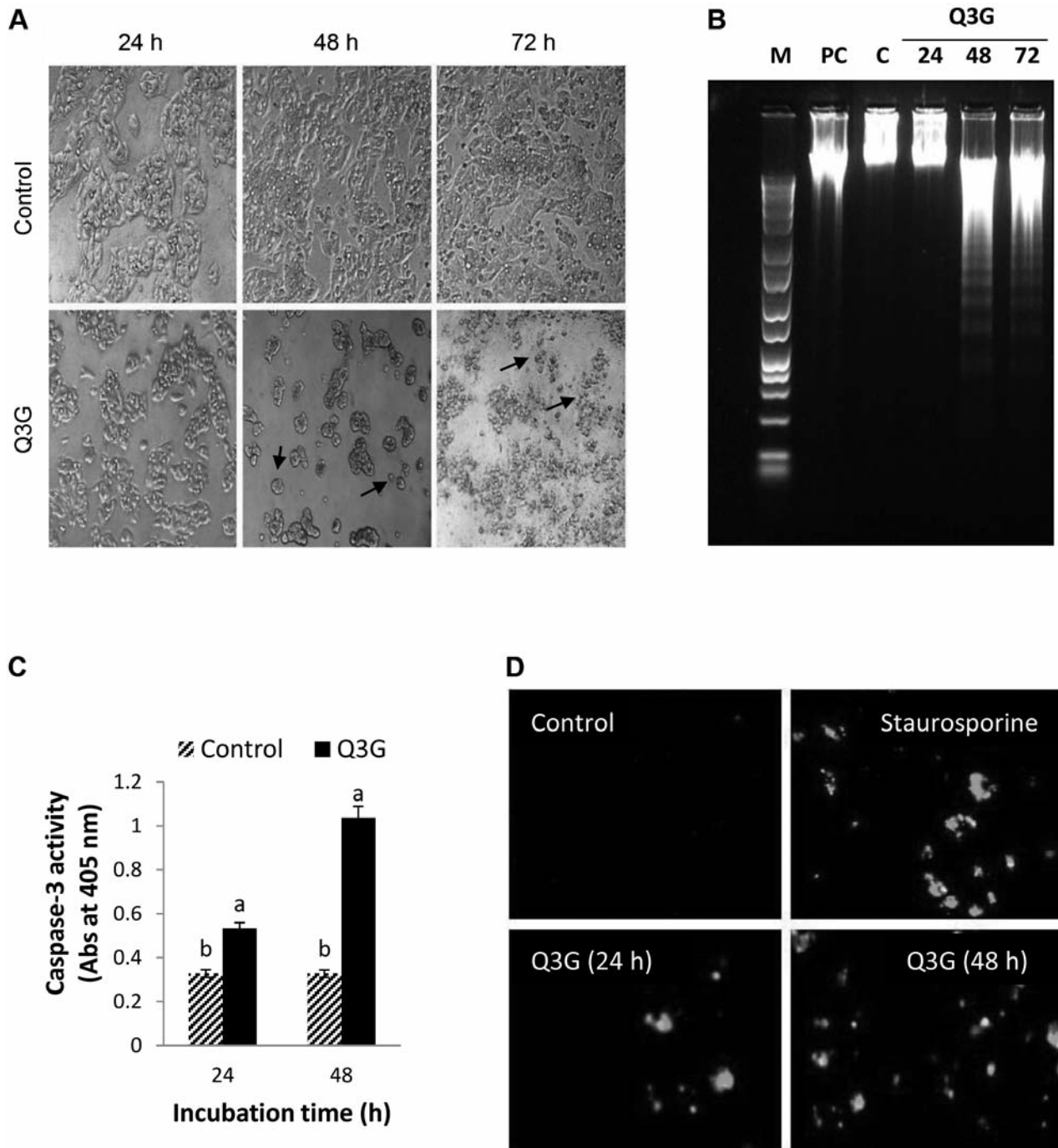


Figure 3. Cell-cycle analysis of quercetin-3-O-glucoside (Q3G)-treated HepG2 cells. HepG2 cells were treated with 100  $\mu$ M Q3G as indicated and then incubated at 37°C for 24 h. Cells were harvested and fixed in 70% alcohol and then stained with propidium iodide. Stained cells were analyzed for cell-cycle phase distributions using a flow cytometer. A: Representative DNA histograms of the flow cytometric analysis are shown for control and each treatment. B: The percentage of cells in G<sub>1</sub>, S, and G<sub>2</sub>/M phases was calculated and is summarized as a bar graph of the mean values (n=3).

manufacturer's instruction. Briefly, HepG2 cells ( $1 \times 10^6$  cells/well) were plated in six-well tissue culture plates. After treatment with 100  $\mu$ M Q3G for 24 and 48 h, the cells were lysed with lysis buffer provided by the manufacturer and centrifuged at 13,000 rpm. The

supernatant was collected. The protein was quantified with BCA protein quantification kit using 250  $\mu$ g of protein for the assay. After the addition of reaction buffer and DEVD-pNA (Asp-Glu-Val-Asp *p*-nitroanilide) as caspase substrate, the microplate was incubated at



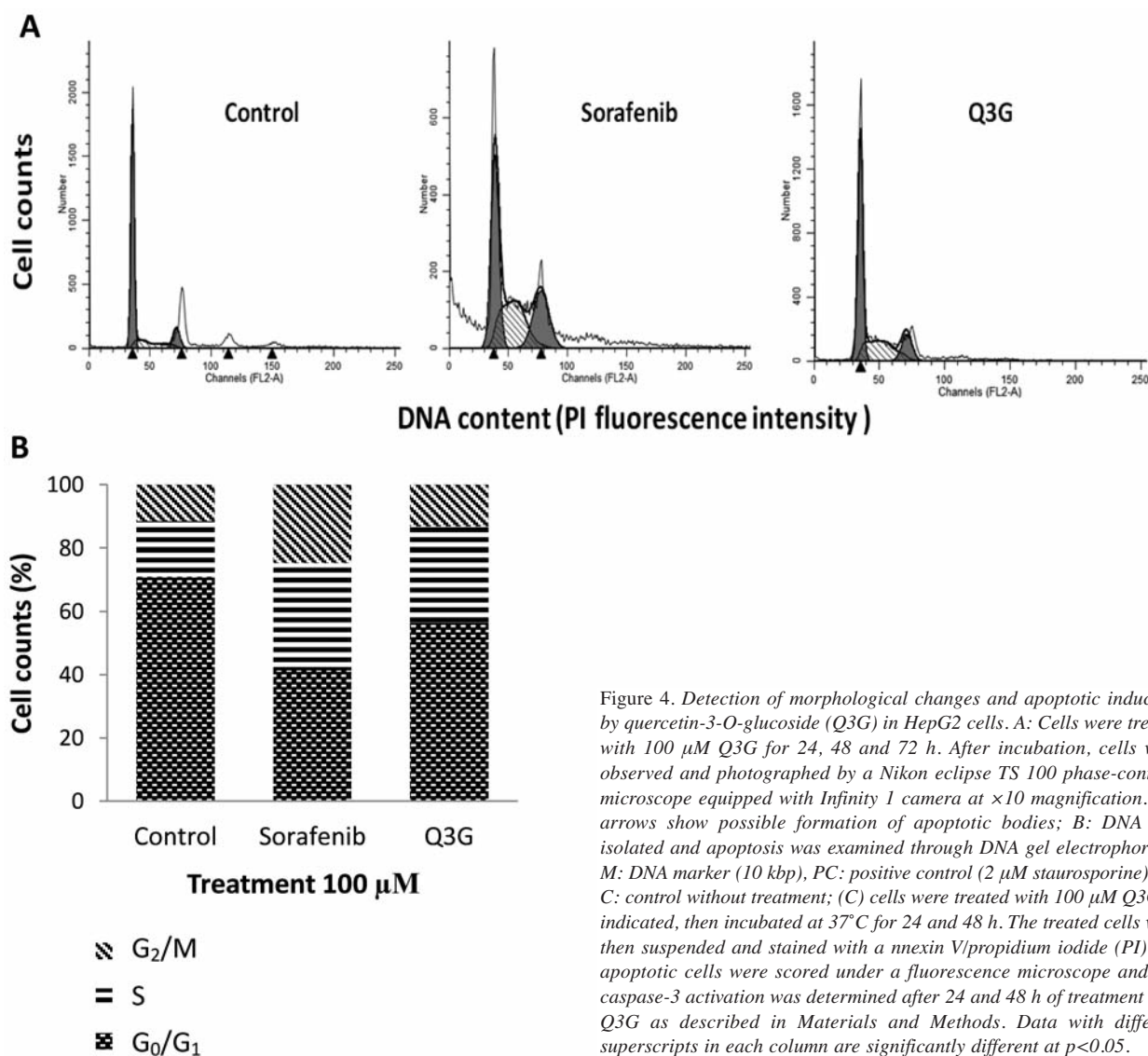


Figure 4. Detection of morphological changes and apoptotic induction by quercetin-3-O-glucoside (Q3G) in HepG2 cells. A: Cells were treated with 100  $\mu$ M Q3G for 24, 48 and 72 h. After incubation, cells were observed and photographed by a Nikon eclipse TS 100 phase-contrast microscope equipped with Infinity 1 camera at  $\times 10$  magnification. The arrows show possible formation of apoptotic bodies; B: DNA was isolated and apoptosis was examined through DNA gel electrophoresis, M: DNA marker (10 kbp), PC: positive control (2  $\mu$ M staurosporine) and C: control without treatment; (C) cells were treated with 100  $\mu$ M Q3G as indicated, then incubated at 37°C for 24 and 48 h. The treated cells were then suspended and stained with a nnein V/propidium iodide (PI) and apoptotic cells were scored under a fluorescence microscope and (D) caspase-3 activation was determined after 24 and 48 h of treatment with Q3G as described in Materials and Methods. Data with different superscripts in each column are significantly different at  $p < 0.05$ .

37°C for 1-2 h. The absorbance of the samples was read at 405 nm in a FluoStar Optima microplate reader (BMG Labtech). Fold-increase in CPP32 activity was determined by comparing the absorbance of lysate for Q3G-treated cells with that of the uninduced control lysate.

**Measurement of DNA topoisomerase II activity.** Commercially-available topoisomerase II drug screening kit (TopoGEN, Inc., Columbus, OH, USA) was used and the assay was performed as per the manufacturer's instructions. Briefly, substrate supercoiled pHot1 DNA (0.25  $\mu$ g) was incubated with 4 units (2  $\mu$ l) of human DNA topoisomerase II, 100  $\mu$ M Q3G (2  $\mu$ l) and assay buffer (4  $\mu$ l) at 37°C for 30 min. The reaction was terminated by the addition of 10% sodium dodecyl sulphate (2  $\mu$ l) followed by digestion with proteinase K (50  $\mu$ g/ $\mu$ l) at 37°C for 15 min. DNA was run on 1% agarose gel in BioRad gel electrophoresis system for 1-2 h and then

was stained with GelRed™ stain for 2 h followed by destaining for 15 min with TAE buffer. The gel was imaged via BioRad's Gel Doc™ EZ system. Supercoiled DNA and linear strand DNA were incorporated in the gel as markers for DNA topology and DNA topoisomerase II poison (inhibitor). The inhibitory activity was calculated as relative inhibition of relaxation activity of topoisomerase enzyme in the presence of Q3G in comparison to that in the absence of Q3G.

**Fluorescence microscopy.** For detecting apoptosis, green fluorescent protein (GFP)-Certified™ Apoptosis/Necrosis Detection Kit was purchased from Enzo Life Sciences Inc. (Farmingdale, NY, USA) and used for the assay. Briefly,  $2 \times 10^5$  HepG2 cells were seeded on two-well chambered coverslips followed by treatment with 100  $\mu$ M Q3G for 24 h. Staining was performed according to the manufacturer's instructions by dual detection reagent [containing

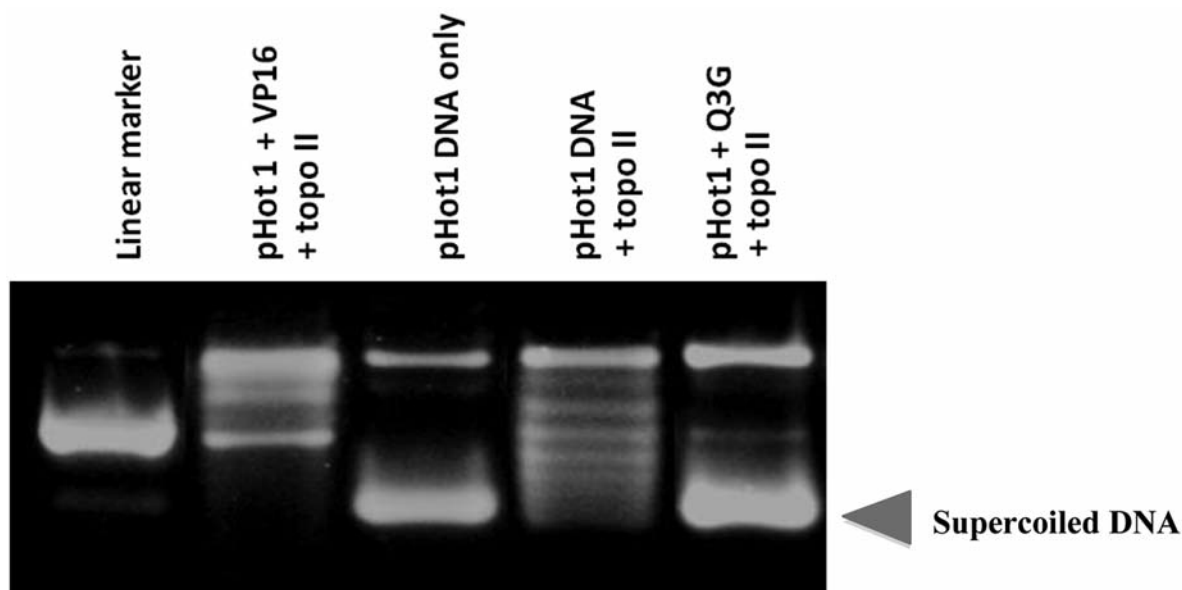


Figure 5. *Quercetin-3-O-glucoside (Q3G)-induced DNA topoisomerase II inhibition.* To assess the effect of Q3G on DNA topoisomerase II DNA relaxation activity, supercoiled circular pHot1 DNA (0.25  $\mu$ g) was taken in test and control tubes and incubated with 4 units of the DNA topoisomerase II enzyme. The reactions were kept at 37° C for 30 min followed by 1% agarose gel electrophoresis. The gel was then stained with GelRed™ staining for 2 h and then destained with TBE buffer for 15 to 20 min. The gel was imaged in Bio-Rad Gel doc system. Lane 1, Linear DNA marker; lane 2, pHot1 DNA + positive control drug VP16 (etoposide) + topoisomerase II; lane 3, supercoiled pHot1 DNA; lane 4, pHot1 DNA+topoisomerase II; lane 5, pHot1 DNA+100  $\mu$ M Q3G+topoisomerase II. DNA topoisomerase inhibition was detected by comparing the ability of DNA topoisomerase II to relax the supercoiled DNA in the presence and absence of Q3G.

annexin V coupled with propidium iodide, (PI)]. The dual-labeled cells were visualized by fluorescence microscopy with a Leica DMBL ( $\times 20/0.040$ ) fluorescent microscope (Houston, TX, USA) attached to a Nikon CoolPix 4500 digital camera (Nikon). Cells with bound annexin-V exhibit green staining in the plasma membrane; cells that have lost membrane integrity exhibit red staining (PI) throughout the cytoplasm, with an impression of green staining on the plasma membrane. Cells with green staining were scored as apoptotic, cells with both green and red staining were scored as late apoptotic, whereas those with only red staining were considered necrotic.

**Cell cycle analysis.** HepG2 cells were cultured in six-well culture plate ( $1 \times 10^6$  cells/well) for 24 h under standard growth conditions. The cells were then treated with 100  $\mu$ M Q3G for a further 24 h. Cells were trypsinized and centrifuged at 1200 rpm at 4°C for 10 min followed by PBS wash. The pellet was re-suspended in 0.3 ml of PBS and the cells were fixed by adding 0.7 ml ice-cold ethanol for 2 h. The cells were centrifuged again at 1200 rpm at 4°C for 10 min and the cell pellet was re-suspended in 0.25 ml of PBS with the addition of 5  $\mu$ l of 10 mg/ml RNase A and incubation at 37°C for 1 h. After incubation, 10  $\mu$ l of 1 mg/ml PI solution was added to the cell suspension and kept in the dark at 4°C until analysis. The cells were then analyzed for cell cycle distribution using a FACS Caliber flow cytometer (BD Biosciences, San Jose, CA, USA) with an excitation wavelength at 488 nm and emission at 670 nm. DNA content was determined by MotFit LT™ software, version 4.0

(Topsham, ME, USA), which provided histograms to evaluate cell cycle distribution.

**Statistical analysis.** Unless otherwise indicated, results are expressed as the mean $\pm$ SD from at least three independent experiments. Data were analyzed using Minitab 16 statistical software. Group differences were analyzed using one-way ANOVA. Tukey's test was performed for highlighting significant differences between different treatment groups. Differences were considered statistically significant at  $p < 0.05$  in all assays.

## Results

**Inhibition of HepG2 cell growth.** The effect of Q3G treatment on the growth of HepG2 cell lines is shown in Figure 1A. The cell growth decreased in a dose-dependent manner ( $p < 0.05$ ) after 24, 48 and 72 h of treatment with Q3G. At higher concentration (200  $\mu$ M), 24-h treatment led to 50% inhibition of cell growth; however, lower concentrations (50 and 100  $\mu$ M) led to up to 98% inhibition after 48 and 72 h ( $p < 0.05$ ). Relevant results were shown by LDH cytotoxicity assay. After treatment with 100  $\mu$ M Q3G, cell membrane integrity decreased in a time-dependent manner, reaching 93% cell damage after 48 h of treatment (Figure 1B).

**Q3G-induced toxicity in primary cell lines.** To evaluate and compare the toxicity caused by Q3G towards normal cells, 100  $\mu$ M Q3G and sorafenib were incubated with NHEPS, RTCP-10 and WI-38 cells for 24 and 48 h and MTS assay was performed. The results showed a significantly higher viability of all three primary cell lines when treated with Q3G compared to sorafenib at the same concentration. This result showed that Q3G exhibited significantly lower cytotoxicity than sorafenib ( $p < 0.05$ ) towards all three primary cell lines tested (Figure 2).

**Q3G blocked cell cycle in S-phase.** To determine the cellular mechanism of growth inhibition of Q3G in HepG2 cells, we investigated cell-cycle progression after Q3G treatment. When compared to the control, treatment with 100- $\mu$ M Q3G significantly increased the population of cells in the S phase with a corresponding decrease of cells in the  $G_1/G_0$  phase after 24 h treatment (Figure 3), implying that DNA synthesis was hindered. On the other hand, sorafenib led to an S/ $G_2$ -M phase arrest, which was consistent with the previous study (32).

**Q3G induced apoptotic cell death through caspase-3 activation.** Treatment with 100  $\mu$ M Q3G for 24, 48 and 72 h led to severe changes in the morphology of HepG2 cells as observed through phase-contrast microscopy (Figure 4A). To examine whether the changes were due to apoptosis, basic apoptotic hallmark assays were performed. Treatment with 100  $\mu$ M Q3G showed clear DNA fragmentation (Figure 4B) and increase in caspase-3 activity (Figure 4C) in HepG2 cells in a time-dependent manner. To further understand the differentiation between apoptosis and necrosis, the cells were examined by annexin V and PI staining after treatment for 24 h with 100  $\mu$ M Q3G *via* fluorescent microscopy. Figure 4D shows representative images of Q3G-treated and untreated HepG2 cells. The untreated cells did not show any staining, suggesting that these cells did not undergo significant apoptosis or necrosis. Few Q3G-treated cells showed staining for both annexin V and PI (yellow), which reflects late apoptotic cells, and for PI only (red), which denotes necrotic cells. Comparatively more cells showed staining for annexin V-only (green), which signifies apoptotic cells (Figure 4D).

**Q3G Induced DNA topoisomerase II inhibition.** To assess if the induction of apoptosis was a result of DNA topoisomerase II inhibition, we examined the catalytic activity of DNA topoisomerase II upon treatment with Q3G *via* gel electrophoresis, as described in Materials and Methods. A representative gel image of the relaxation assay for determining the catalytic inhibition on human DNA topoisomerase II with controls is presented in Figure 5. The assay was performed to test whether Q3G acts as a poison and increases DNA cleavage *via* topoisomerase II. As shown

in Figure 5, Q3G did not stabilize topoisomerase II cleavage complexes and failed to induce the formation of linear DNA, and increased the supercoiled DNA intensity, whereas the positive control drug VP-16 increased the formation of linear DNA. This shows that Q3G does not act as a human topoisomerase II poison but as a catalytic inhibitor by inhibiting DNA relaxation activity.

## Discussion

In the present study, we found that Q3G, a common flavonoid present in fruits, can significantly inhibit proliferation of HepG2 cells. Proliferation of HepG2 cells was shown to decrease in a dose- and time-dependent fashion. To the best of our knowledge, this is the first study to show the time-dependent response of Q3G treatment on cell proliferation and its mechanism of action in HepG2 cells. Fruit extracts containing Q3G have been shown to inhibit cell proliferation against various cancer cell lines (20-22). Synergistic effect of Q3G has also been shown to improve the anti-proliferative properties of apple extracts (19). Additionally, Q3G has been shown to exhibit stronger anti-proliferative action than its aglycone quercetin, possibly due to the presence of a glucose moiety favoring better absorption of Q3G (18). This makes Q3G an important compound for cancer therapeutics as far as bioavailability is concerned. Studies have previously shown various side-effects of cancer drugs, such as sorafenib, which limit the efficacy of these drugs in treatment (27). Our data demonstrated that Q3G treatment was comparatively less toxic towards primary normal cell lines such as NHEPS, RTCP-10 and WI-38 cells than sorafenib at the same concentration. This explains the specific action of Q3G on cancer cells. Consistent with previous studies, our data reveal that Q3G exhibits a strong anti-proliferative action on HepG2 cells with an increase in treatment time.

The inhibitory effect of Q3G on the proliferation of HepG2 cells may effect two cellular mechanisms, cell-cycle arrest and induction of apoptosis. Our investigation of the cell-cycle distribution of Q3G-treated HepG2 cells revealed that the cell-cycle was arrested in the S phase upon 100  $\mu$ M Q3G treatment of cells. Previous studies have shown that quercetin can induce cell-cycle arrest not only in the S phase (23) but also in the  $G_1/S$  phase (25) or  $G_2/M$  phase (24), depending on the cancer cell type. In HepG2 cells, quercetin induced  $G_1$  phase arrest (26). Relevantly, in our study, Q3G prevented HepG2 cells from entering  $G_2$  phase, resulting in accumulation of cells in the S phase. This explains why the cell-cycle regulation is cell-type and treatment-dependent.

Induction of apoptosis is another possible mechanism by which the antiproliferative activity of Q3G in HepG2 cells may be exerted. In the current study, we demonstrated that HepG2 cells articulate an apoptotic reaction upon Q3G

treatment. The evidence included annexin V staining analysis, activation of caspase-3 in Q3G-treated HepG2 cells and Q3G-induced inhibition of DNA topoisomerase II. Aglycone quercetin has been shown to induce apoptosis *via* activation of caspases in HepG2 cells (28). Extracts containing Q3G have also shown to increase caspase-3 activity (22). Consistent with these results, our data showed a significant activation of caspase-3 in HepG2 cells upon treatment with Q3G increasing with increasing incubation time. Apoptosis has been shown to be the most efficient cell death pathway in tumor cells after topoisomerase II inhibition (29). Aglycone quercetin has been shown to inhibit DNA topoisomerase activity II (30). Additionally, extracts containing flavonol quercetin glycosides have been shown to inhibit both topoisomerase I and II in HepG2 cells, resulting in cell death (31). Interestingly, consistent with these studies, our data showed that Q3G strongly inhibited DNA topoisomerase II activity, which may be a possible reason for apparent cell death and apoptosis. It should be pointed-out that these effects may or may not be specific to HepG2 cells. Overall, our study suggests that Q3G possesses a strong specific anti-proliferative activity towards HepG2 cells and is therefore a potential candidate for liver cancer chemotherapy. However, additional studies need to be performed in order to further understand the specific upstream factors mediating the Q3G induction of cell cycle arrest and apoptosis.

## Conflicts of Interest

None.

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