Original Research Article **Cell biology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

ANTI-CANCER ACTIVITY OF QUERCETIN, GALLIC ACID, AND ELLAGIC ACID AGAINST HEPG2 AND HCT 116 CELL LINES: *IN VITRO*

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ABSTRACT

Hepatocarcinoma (HCC) and colorectal cancer (CRC) are the most malignant tumors with increasing incidences of morbidity and mortality globally.The aim of the present study was to determine *in vitro* anti-tumor activity of quercetin, gallic acid, and ellagic acid against HCC and CRC. This was achieved through testing the effects of these compounds on HepG2 and HCT 116 cells, ranging from cell growth inhibition assay and fold dose advantage, passing by mitochondrial activity assessment, to cellular protein content measurement. The efficacies of these compounds were compared with doxorubicin in case of HepG2 cells and 5-fluorouracil in case of HCT 116 cells. The present findings indicated that in both cell types, gallic acid elicited the most pronounced anti-cancer activity and mitochondrial dysfunction compared to quercetin and ellagic acid. Extensive research is warranted to assess the precise molecular mechanisms of these natural products in bridling HCC and CRC.

KEYWORDS: quercetin, gallic acid, ellagic acid, HepG2, HCT 116, mitochondrial activity.

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INTRODUCTION

Cancer is an uncontrolled cell growth that may invade surrounding tissues and migrate to other parts of the body.[1] Hepatocarcinoma (HCC) is one of the most malignant tumors with a high mortality globally.[2] Meanwhile, colorectal cancer (CRC) has been steadily increasing with high incidences in developing nations.[3] Nowadays, thus natural products have been receiving increased attention in research work and clinical studies in the field of cancer as a result of the remarkable adverse effects of the currently available chemotherapy.[4,5] The potential effects of these products may ultimately suppress cancer growth, angiogenesis, and metastasis without any side effects.[6] Quercetin has been found to cause cell cycle arrest in different cancer cells, as it can modulate some of signaling cascades in some transduction pathways associated with apoptosis (caspases 3, caspases 9, and B-cell lymphoma 2 "Bcl-2") and cell survival (mitogen-activated protein kinase "MAPK" and protein kinase B "PKB", also known as "Akt") processes in acute myeloid leukemia and human gastric cancer cells.[7-8] Therefore, quercetin may be a good chemopreventive and therapeutic agent in cancer.[9] Gallic acid has strong antioxidant anti-inflammatory and anti-cancer properties. In presence of metal ions, it has pro-oxidant property in concentration dependent manner which induces the apoptotic signaling pathway in cancerous cells.[10] Moreover, it has been revealed that matrix metalloproteinase-2 (MMP-2) and MMP-9 proteolytic activities were inhibited *via* gallic acid.[11] Ellagic acid has been found to have strong anticancer activity.[12] 3,3'-Di-O-methyl ellagic acid-4'-O-β-d-xylopyranoside (JNE2), an ellagic acid derivative, could inhibit the proliferation of HepG2 cells.[13] Specifically, JNE2 inhibited the proliferation of HepG2 cells and blocked cell cycle at the G1/S phase *via* the down regulation of G1/S-specific cyclin-D1 (CCND1). A high dosage of JNE2 persuaded apoptosis of the cancer cells through upregulation of the protein expression of Bcl-2-associated X protein (Bax) and caspase-3, and downregulation of the protein expression of Bcl-2, which is a key regulator of apoptosis that associated with the mitochondrion.[14-15] Therefore, it was dedicated that JNE2 acts on controlling of this apoptotic ratio between Bcl-2 and Bax genes.[13] This study was planned to explore the anti-cancer activity of quercetin, gallic acid, and ellagic acid *in vitro*. Cytotoxic capacity, mitochondrial activity, and cellular protein contents have been evaluated for HepG2 and HCT116 treated cells versus untreated cells. Also, the efficacies of these compounds were compared with doxorubicin and 5-fluorouracil in case of HepG2 and HCT 116 cells respectively.

MATERIALS AND METHODS

Cell lines

Human hepatocellular carcinoma and colorectal cell lines (HepG2 and HCT 116 cells) were purchased from VACSERA (Egypt) which purchased them from American Type Culture Collection (ATCC, USA).

Compounds and chemicals

Quercetin≥95% (HPLC), solid (Q4951), gallic acid 97.5-102.5% (titration) (G7384), ellagic acid 95% (HPLC) powder, Roswell Park Memorial Institute Medium (RPMI-1640), Dulbecco's Modified Eagle Medium (DMEM), L-glutamine, Doxorubicin (DOX), 5-fluorouracil (5-FU), dimethyl sulfoxide (DMSO), and 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) were purchased from (Sigma, St Louis, MO, USA).Trypsin-EDTA was purchased from (Lonza, USA). Fetal calf serum and penicillin/streptomycin were purchased from (Wexford, Ireland). Sulphorhodamine-B (SRB) was purchased from Duchefa-Biochemie (Haarlem, Amsterdam, Netherlands). All reagents and chemicals used for analysis met the quality criteria in accordance with international standards.

In vitro studies

Cell propagation and maintenance

HepG2 cells were propagated in the proper conditions (at 37ºC and 5% CO_2) and maintained in RPMI-1640 with 1% L-glutamine and supplemented with 10% fetal calf serum for growth and 1% penicillin/streptomycin. Meanwhile, HCT 116 cells were cultured and propagated in a C-DMEM. When the cells are approximately 80% confluent, they were subcultured using Trypsin-EDTA. Second round of HepG2 and HCT 116 cells subculturing was used for 96-well plates seed in gand thereafter drug screening.

Growth inhibition assay

The cytotoxic effects of the pure compounds (quercetin, gallic acid, and ellagic acid) as well as the standard anti-cancer drugs, doxorubicin (DOX) and 5-fluorouracil (5-FU), at the concentrations (0, 6.25, 12.5, 25, 50 and 100 µM) were investigated on HepG2 and HCT 116 human cancer cell lines using the sulphorhodamine-B (SRB) assay.[16] Briefly, the cells were seeded in 96 well microtiter plates at a concentration of 5000 cells/well and left for cell attachment on the plate for 24 h in 5% CO_2 at 37°C. After 24 h, cells were incubated for 48 h with various concentrations of the pure compounds as well as the standard anti-cancer drugs (0, 6.25, 12.5, 25, 50 and 100 µM).Following 48 h treatment, the medium were discarded, the cells were fixed with 10% trichloroacetic acid (TCA) 150 µl/well for 1 h at 4°C (TCA reduce SRB protein binding). Then, the cells were washed with distilled water 3 times. Wells were stained for 30 min at room temperature with 50 µL of 0.4% SRB dissolved in 1% acetic acid at room temperature (25 ± 2°C) and kept in dark place. After incubation, the SRB solution was poured off and the plates were washed with 1% acetic acid to remove unbound dye and to leave only the cell adhered dye. Then, the plates were air dried and the dye was solubilized with 150 µl/well of 10 mM tris base solution (PH 7.4), and the mixture was shaken for 5 min at room temperature (25 ± 2°C). The optical density (OD) of each well was measured spectrophotometrically

at 545 and 600 nm with an ELISA microplate reader. The experiment was performed in triplicate and the percentages of cell viability were calculated.

The half inhibitory concentration (IC_{50}) and fold change

The half maximal inhibitory concentration (IC_{50}) values representing the concentrations that inhibit 50% of cell viability were obtained by plotting the percentages of cell viability versus the concentrations of the sample using polynomial concentration–response curve fitting models (Origin Pro 8 Software). Finally, the fold change of compounds (quercetin, gallic acid, and ellagic acid) versus DOX in case of HepG2 cells and 5-FU in case of HCT 116 cells were calculated as well.

Mitochondrial activity measurements

The 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) from (Sigma, St Louis, MO, USA) is based on the conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity.[17] The effects of the studied compounds as well as the standard anti-cancer drugs (DOX and 5-FU) on the mitochondrial activity were estimated by MTT assay using HepG2 and HCT 116 cells. Briefly, the cells were cultured in 96-well plates at a density of 1×10^4 cells/well. 0, 25, 50, 100 µM of the compounds as well as DOX and 5-FU were added per well in RPMI-1640 (HepG2 cells) and DMEM (HCT 116 cells). Also, media without drug was used as control. After 1 day incubation, MTT dissolved in PBS was added to each well at a final concentration of 5 mg/ml, and the samples were incubated at 37°C for 4 h. Water-insoluble crystals of formazan that formed during MTT cleavage in actively metabolizing cells were then dissolved in dimethyl sulfoxide (DMSO) using 100 µl/well. Absorbance was measured at 455 nm, using a microplate reader (Model 500; BIORed Instrument Inc., USA). The mitochondrial activity (%) was calculated and compared with the control.

Cellular protein measurements

The protein content of HepG2 and HCT 116 cells upon treatments with the tested compounds, DOX, and 5-FU was measured according to the previously optimized method.[18] Basically, sulforhodamine B, the protein dye, binds electrostatically and pH dependent on protein basic amino acid residues of trichloroacetic acid-fixed HepG2 and HCT 116 cells. After incubation with the compounds, cell monolayers are fixed with 10% (wt/vol) trichloroacetic acid and stained for 30 min with SRB, after which the excess dye is removed by washing repeatedly with 1% (vol/vol) acetic acid. The protein-bound dye is dissolved in 10 mM Tris base solution and observed at 510 nm using a microplate ELISA reader.

Statistical analysis

The results were presented as the mean of three independent experiments and the standard deviation (SE). One way analysis of variance (ANOVA) was used for the analysis of the test results at the significance level of *p*-value <0.05. The IC_{50} was obtained using polynomial concentration–response curve fitting models (Origin Pro 8 software).

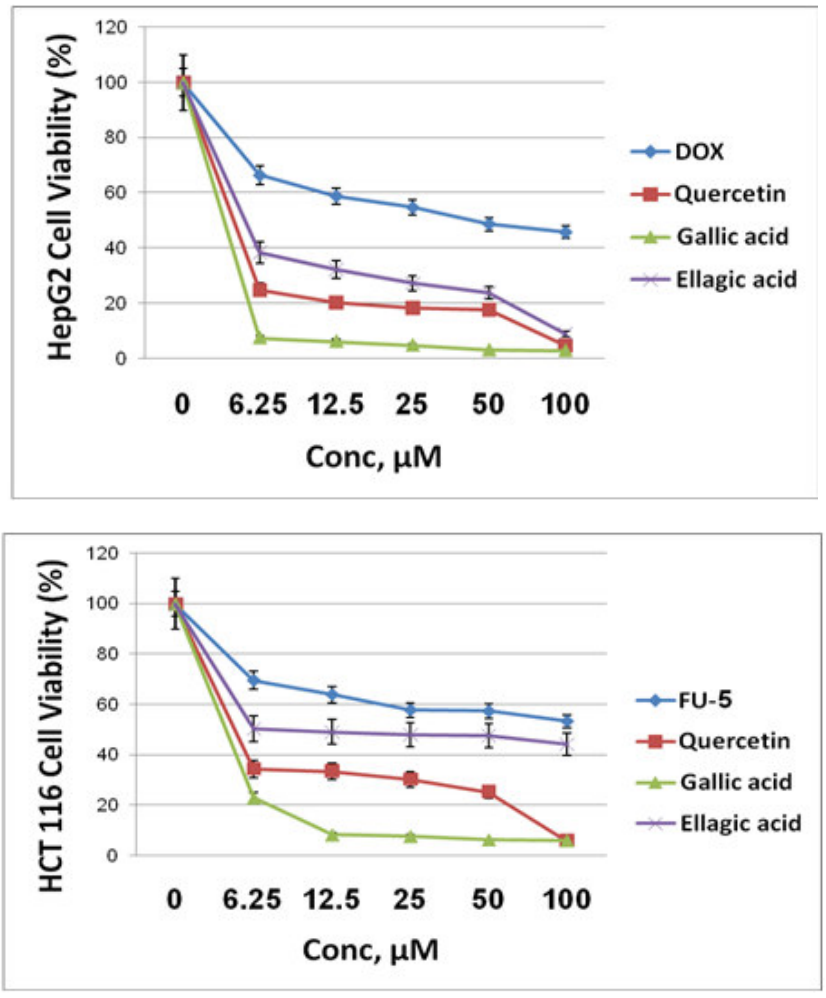
RESULTS

HepG2 cells inhibition

The cytotoxicity of each compound as well as the standard anti-tumor drug; doxorubicin (DOX) was investigated against liver cancer HepG2 cell line at different doses (0, 6.25, 12.5, 25, 50 and 100 µM) using SRB technique. Data illustrated in (Fig. 1a) show the viability percentage of HepG2 cells after 48h from treatment with different concentrations of the tested compounds versus controls. These data revealed that the treatment of HepG2 cells with 6.25 µM of DOX significantly decreased cell viability to 66.43% ($P < 0.01$), whereas cells treated with 12.5 and 25 µM of doxorubicin showed significant decrease in cell viability to 58.87% and 54.80% respectively ($P < 0.01$). Furthermore, treatment of HepG2 cells with 50 and 100 µM of DOX produced significant decrease in cell viability to 48.77% and 45.83%, respectively ($P < 0.01$). These results revealed a dose dependent effect in cell growth of HepG2 cells after DOX treatment, with an IC_{50} value equal 33.06 µM at 48 h (Fig. 2a). On the other side, HepG2 cells treated with 6.25, 12.5 and 25 µM of quercetin showed after 48h significant decrease in cell viability to 25.05%, 20.52% and 18.57% respectively ($P < 0.01$). While, treatment of cells with 50 and 100 µM of quercetin caused significant decrease ($P < 0.01$) in cell viability to 17.96% and 4.91% respectively (Fig. 1a). These data suggested a dose dependent effect in cell proliferation of HepG2 cells after quercetin treatment, with an IC_{50} value equal 7.10 µM at 48 h (Fig. 2a) and fold dose advantage of quercetin versus DOX equal 4.65 (Fig. 2b).Treatment of HepG2 cell line with 6.25 µM of gallic acid significantly decreased cell viability to 7.65% ($P < 0.01$); whereas, cells treated with 12.5 and 25 µM of gallic acid showed significant decrease in cell growth to 6.37% and 4.98% respectively ($P < 0.01$). In addition, treatment of HepG2 cell line with 50 and 100 µM of gallic acid produced significant decrease ($P < 0.01$) in cell viability to 3.22% and 3.01% respectively (Fig. 1a). These observations revealed a dose dependent decrease in HepG2 cell viability after treatment with gallic acid, with an IC_{50} value equal 2.60 µM at 48 h (Fig. 2a) and fold dose advantage of gallic acid versus DOX equal 12.67 (Fig. 2b). At the same line, liver cancer HepG2 cells treated with 6.25, 12.5 and 25 µM of ellagic acid showed after 48h significant decrease ($P < 0.01$) in cell viability to 38.55%, 32.46% and 27.40% respectively. Whilst, treatment of cells with 50 and 100 µM of ellagic acid caused significant decrease ($P < 0.01$) in HepG2 cell viability to 23.93% and 9.07% respectively (Fig. 1a). These results showed a dose dependent decrease in HepG2 cell viability after ellagic acid treatment, with an IC_{50} value equal 11.86 µM at 48 h (Fig. 2a) and fold dose advantage of ellagic acid versus DOX equal 2.78 (Fig. 2b).

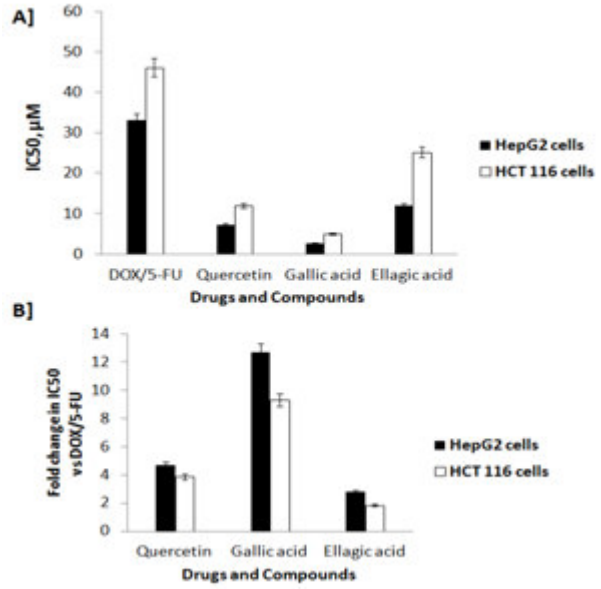
HCT 116 cells inhibition

The cytotoxicity of each compound under study as well as the commercially available drug; 5-Fluorouracil (5-FU) was investigated against colorectal cancerous HCT 116 cell line at concentrations (0, 6.25, 12.5, 25, 50 and 100 µM) using SRB method. Data illustrated in (Fig. 1b) show the percentage of viability of colon cancer HCT 116 cells 48h post-treatment with different concentrations of the tested compounds versus controls. These observations revealed that treatment of HCT 116 cells with 6.25 and 12.5 µM of 5-FU significantly decreased cell viability to 69.62% and 64% respectively ($P < 0.05$). Whereas, cells treated with 25, 50 and 100 µM of 5-FU showed significant decrease in cell viability to 57.87%, 57.37% and 53.37%, respectively ($P < 0.01$). These results revealed a gradual dose decrease in HCT 116 cell viability post-treatment with 5-FU, with an IC_{50} value equal 46.13 µM at 48 h. At the same time, HCT 116 cells treated with 6.25, 12.5 and 25 µM of quercet in showed after 48h significant decrease in cell viability to 34.44%, 33.58% and 30.38% respectively ($P < 0.01$). While, treatment of cells with 50 and 100 µM of quercetin caused significant ($P < 0.01$) decrease in HCT 116 cell viability to 25.41% and 5.85% respectively (Fig. 1b). These data showed a gradual dose decrease in HCT 116 cell viability after quercetin therapy, with an IC_{50} value equal 11.94 µM at 48 h (Fig. 2a) and fold dose advantage of quercetin versus 5-FU equal 3.86 (Fig. 2b). Treatment of HCT 116 cells with 6.25 µM of gallic acid significantly decreased ($P < 0.01$) cell viability to 22.87% whereas cells treated with 12.5 and 25 µM of gallic acid showed significant decrease ($P < 0.01$) in cell viability to 8.19% and 7.63% respectively. Likewise, treatment of HCT 116 cells with 50 and 100 µM of gallic acid produced significant ($P < 0.01$) decrease in HCT 116 cell viability to 6.29% and 5.93% respectively (Fig. 1b). These results revealed a dose dependent decrease in HCT 116 cell viability of after incubation with gallic acid, with an IC_{50} value equal 4.94 µM at 48 h (Fig. 2a) and fold dose advantage of gallic acid versus 5-FU equal 9.32 (Fig. 2b). On the other side, HCT 116 cells treated with 6.25, 12.5 and 25 µM of ellagic acid showed after 48h significant decrease ($P < 0.01$) in HCT 116 cell viability to 50.45%, 49.20% and 48.0% respectively. Whereas, treatment of cells with 50 and 100 µM of ellagic acid caused significant ($P < 0.01$) decrease in HCT 116 cell viability to 47.83% and 44.27% respectively (Fig. 1b). These observations suggested a gradual dose dependent decrease in HCT 116 cell viability after ellagic acid treatment, with an IC_{50} value equal 25.19 µMat 48 h (Fig. 2a) and fold dose advantage of ellagic acid versus 5-FU equal 1.83 (Fig. 2b).



Figures 1

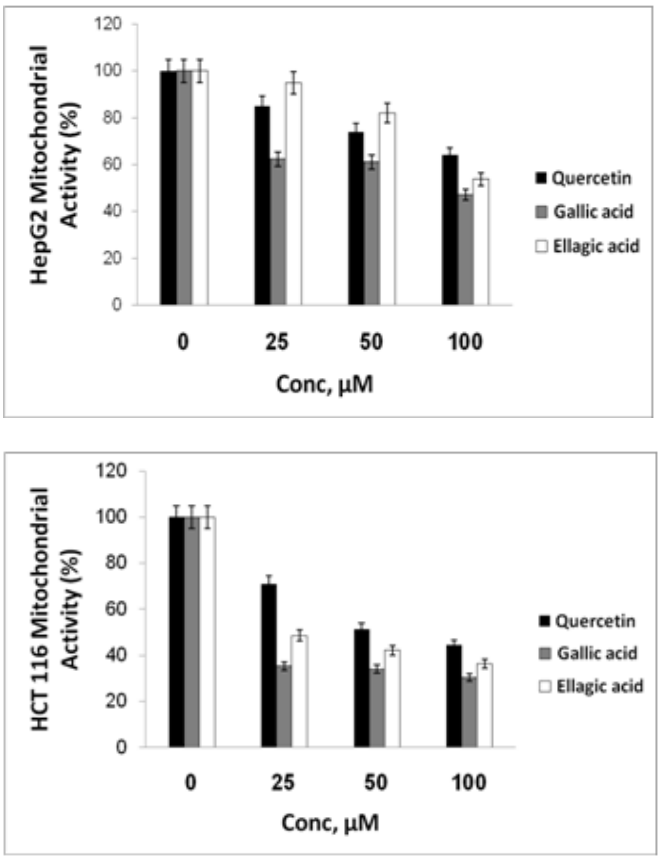
Growth inhibition of HepG2 cells (a) and HCT 116 cells (b) using SRB-based cytotoxic drug screening assays of quercetin, gallic acid, and ellagic acid versus DOX (a) and 5-FU (b) (n=3).



Figures 2

The half maximal inhibitory concentrations (IC_{50}) (a) and fold change (b) of quercetin, gallic acid, and ellagic acid versus DOX in case of HepG2 cells and 5-FU in case of HCT 116 cells (n=3).Mitochondrial activity

The results in Fig. 3, show the effect of quercetin, gallic acid, and ellagic acid versus DOX on mitochondrial activityof human HepG2 cells as well as versus 5-FU on mitochondrial activity of human ofhuman HCT 116 cells. Interestingly, gallic acid, compared to quercetin and ellagic acid, produced remarkably superior effects at 25, 50, and 100 µM ($P < 0.05$). On the other side, quercetin, compared to ellagic acid, reduces the cellular mitochondrial activity of HCT 116 cells at 25, 50, and 100 µM concentrations and HepG2 cells at 100 µM concentration, meanwhile the effect of ellagic acid exceeds quercetin at 25 and 50 µM concentrations.

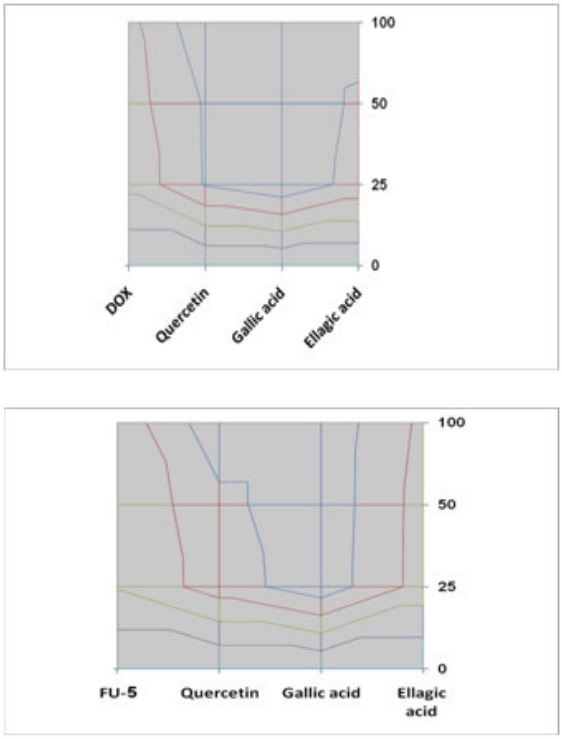


Figures 3

HepG2 (a) and HCT 116 (b) cellular mitochondrial activities using MTT-based assays of quercetin, gallic acid, and ellagic acid (n=3).

Cellular protein content screening

We measured the protein content in HepG2 and HCT 116 treated with the studied compounds, DOX and 5-FU. The lower the cellular protein content, the higher the cytotoxic effect of the compound. This is due to the SRB dye binds to the amino acid residues of the trichloroacetic acid-fixed cells. Hence, we recorded that gallic acid had the lowest cellular protein contents and the highest cytotoxic effect compared to quercetin and ellagic acid against both HepG2 and HCT 116 cells (Fig. 4).



Figures 4

HepG2 (a) and HCT 116 (b) cellular protein contents of quercetin, gallic acid, and ellagic acid versus DOX in case of HepG2 cells (a) and 5-FU in case of HCT 116 cells (b) (n=3). Doses ranging from 0 to 20, 20 to 40, 40 to 60, 60 to 80, and 80 to 100 µM versus quercetin, gallic acid, ellagic acid, and DOX/5-FU were used to illustrate the cellular protein content of either HepG2 or HCT116 cancer cell lines.

DISCUSSION

The current study clarified that gallic acid then quercetin elicited the most pronounced anti-cancer activity and cytotoxic effect on HepG2 and HCT 116 cell lines compared to ellagic acid, DOX, and 5-FU. This is in agreement with the previous studies indicated that gallic acid possesses cytotoxic possessions in several cancer cell lines and can selectively induce cancer-mediated apoptosis without harming healthy cells.[11,19-21] Moreover, the *in vitro* antiproliferative activity of gallic acid from methanol extract of *Morus alba* has been documented to induce cytotoxicity in human colon cancer cell line (HCT-15).[22] Moreover, gallic acid could induce apoptosis and inhibit transcription factors that endorse cell existence and spread. In particular, it could suppress NF-κB, AP-1, STAT-1, and octamer-1 (OCT-1) inhibition which are known to be activated in CRC. In addition, it was found to decrease Caco-2 cell viability, arrest the cell cycle at G0 /G1, and persuade apoptotic cell death. The stimulation of the apoptotic pathway by gallic acid was shown by the triggering of caspase-3 and it also caused DNA destruction and nuclear condensation.[23] Regarding quercetin, aubiquitous bioactive flavonoid, *in vitro* experiments have demonstrated that it constrains cell spread and persuades apoptosis in various types of tumor cells *via* diverse signaling pathways.[24-25] It could induce cell growth inhibition and apoptosis in a variety of cancer cells.[9] Quercetin has a clearly cytotoxic effect on HepG2 cells and it causes a 4- to 5-fold increase of LDH liberation into the culture medium on HepG2 indicating a significant cytotoxicity in HepG2 culture.[26] The anticarcinogenicity of quercetin includes inhibition of cell proliferation through induction of cell cycle arrest and/or apoptosis.[27] Moreover, querecetin has been reported to have generalized growth inhibitory effect as well as antigenotoxic and antiproliferative effects in HepG2 and several other cell lines.[28-29]Mechanistically, various signaling pathways have been suggested for quercetin's anti-tumor activity, including inhibition of glycolysis, up-regulation of cell cycle inhibitors such as p21WAF1 and p27KIP1, and down-regulation of oncogene expression.[30] Also, quercetin could activate AMP-activated protein kinase (AMPK).[31-32] As several studies have been demonstrated that tumor suppressor proteins, including liver kinase B1 (LKB1), tuberous sclerosis 2 (TSC2), and p53, are associated with the AMPK pathway.[33] Therefore, AMPK initiation by quercetin may link with mediating cell cycle/ apoptosis of cancer cells.[31-32] The inhibitory effect of quercetin on NF-κB transcription factor in turn regulates the expression of other proteins that control cell growth as well as cell cycle regulators or anti-apoptotic proteins.[34] It was also verified that mitochondria seems to be a crucial target for quercetin, causing a disruption in the

mitochondrial membrane potential (MMP).[35] NF-κB factor promotes proliferation and cell survival by enhancing the expression of several genes including Bcl-2, B-cell lymphoma 2 extra-large (Bcl-XL), cellular inhibitor of apoptosis (cIAP), survivin and TNF receptor associated factor (TRAF). The proteins coded by those genes actmainly by interfering with one of the apoptotic elements. As a consequence, the natural suppression of NF-κB leads to an abrogation of proliferation which allows cells to come into the death pathways.[34] On the other hand, the present study results showed that ellagic acid enhanced significant cytotoxic effects on HepG2 and HCT 116 cell lines compared to DOX and 5-FU respectively. Ellagic acid, a type of polyphenols that widely exist in herbs, fruits, and nuts, has recently gained increasing attention. It has been well established that ellagic acid exhibits anticancer and antimutagen, as well as others.[36-37] A number of antitumor agents activate mitochondria-arbitrated apoptosis in cancerous cells *via* the downregulation of Bcl-2 and Bcl-xL or the upregulation of Bax, Bad, and Bid. The caspase-3 expression was noticed in HepG2 cells following therapy with an ellagic acid derivative and the upregulated influence was investigated.[13] These results said that it persuaded the apoptosis of human liver cancerous HepG2 cells throughout the mitochondrial pathway. Caspase-3 is elaborated in apoptosis persuaded by Bcl-2/Bax, p38 and Janus-kinase-signal transducer and activator of transcription (JAK-STAT).[38-39] Additionally, it has been revealed that ellagic acid decreases viable cell number of human colon tumor cells.[40] Moreover, ellagic acid has previously been shown to enhance antitumorigenic characterization like induction of apoptotic pathways and cell cycle arrest.[41-42] It has been shown that ellagic acid is a strong antiproliferative, apoptotic and antioxidant influences which may be an appliance whereby they inhibit cancer cell propagation and activate cancer cell death through apoptosis.[40] DOX exhibits significant cytotoxic effect on HepG2 cell line and it could be attributed to its ability to activate c-jun N-terminal kinase (JNK) and CHOP signalling pathways.[43-44] Turning on these stress pathways activates the proapoptotic Bcl-2 family protein "Bim" through elevated gene expression and/or phosphorylation, leading to mitochondrial cell death.[45] Also, 5-FU produced significant cytotoxic effect on HCT 116 cell line. This is expected since 5-FU is known to interfere with the metabolism of nucleoside which can be incorporated into the RNA and DNA. Furthermore, 5-FU inhibits deoxythymidine monophosphate (dTMP) production when converted instead to fluorodeoxyuridine monophosphate to form a stable complex with thymidylate synthase. Since dTMP is essential for DNA replication and repair, inhibition of its production may cause cell death.[46] In accordance with these facts, our data depicted that DOX and 5-FU decreased HepG2 and HCT 116 cells viability, but not as significance as the studied pure compounds. In fact, the natural products can modulate various molecular pathways involved in hepatocellular carcinoma and colon cancers initiation and progression for tackling cancerous cells, while leaving normal cells. It is expected that studies with natural products will define various targets for tumor growth inhibition and apoptosis. To date, chemotherapies in many post-clinical studies with natural compounds directed against hepatic and colorectal cancerous cells are unfortunately very limited. In the present study, gallic acid, quercetin, and ellagic acid proved their activity as cytotoxic mediators against HepG2 and HCT 116 cell lines compared to DOX and 5-FU as standard anticancer agents. Further research is warranted to identify the specificity and target ability, as well as precise molecular mechanisms of these natural compounds against hepatocellular carcinoma and colon cancers, while sparing non-cancerous/normal cells healthy.

ACKNOWLEDGEMENTS

This research study was funded through the project number (10010106) which awarded by the National Research Center.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Rang HP, Dale MM, Ritter JM and Moore P K. Book Review: Pharmacology. London: Churchill Livingstone. Fifth Edition.2003
2. Center MM and Jemal A. International trends in liver cancer incidence rates.Cancer Epidemiology Biomarkers and Prevention. 2011 Nov; 20: 2362–8.
3. Schnekenburger M and Diederich M. Epigenetics Offer New Horizons for Colorectal Cancer Prevention. Curr Colorectal Cancer Rep. 2012 Mar; 8(1):66-81.
4. Abd-Rabou AA, Zoheir K, Ahmed HH. Potential impact of curcumin and taurine on human hepatoma cells using huh-7 cell line. Clinical biochemistry. 2012 Jul; 45: 1519-21.
5. Harlev E, Nevo E, Ephraim P, Lansky EP, Lansky S, Bishayeed A. Anticancer attributes of desert plants: a review. Anticancer Drugs. 2012 Mar; 23: 255-71.
6. Aggarwal BB and Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. Biochem. Pharmacol.2006 May; 71:1397-421.
7. Kaneuchi M, Sasaki M, Tanaka Y, Sakuragi N, Fujimoto S and Dahiya R. Quercetin regulates growth of Ishikawa cells through the suppression of EGF and cyclin D1. Int J Oncol.2003 Jan; 22: 159–64.
8. Chang J, Hsu Y, Kuo P, Kuo Y, Chiang L and Lin C. Increase of Bax/ Bcl-XL ratio and arrest of cell cycle by luteolin in immortalized human hepatoma cell line. Life Sci. 2005 Mar; 76: 1883–93.

9. Seufi AEM, Ibrahim SS, Elmaghraby TK and Hafez EE. Preventive effect of the flavonoid, quercetin, on hepatic cancer in rats via oxidant/antioxidant activity: molecular and histological evidences. Journal of Experimental & Clinical Cancer Research. 2009 Jun; 28:80
10. Verma S, Singh A and Mishra A. Gallic acid: Molecular rival of cancer. Environmental Toxicology and Pharmacology. 2013 May; 35: 473–85.
11. Filipiak K, Hidalgo M, Silvan JM, Fabre B, Carbajo RJ, Pineda-Lucena A, Ramos A, Pascual-Teresa B and Pascual-Teresa S. Dietary gallic acid and anthocyanin cytotoxicity on human fibrosarcoma HT1080 cells. A study on the mode of action. Food Funct. 2014 Feb; 5(2):381-9.
12. Thresiamma KC, George J and Kuttan R. Protective effect of curcumin, ellagic acid and bixin on radiation induced genotoxicity. J Exp Clin Cancer Res. 1998 Dec; 17: 431-434.
13. Zhang H, Guo ZJ, XU WM, YOU XJ, HAN L, Han YX and Dai LJ. Antitumor effect and mechanism of an ellagic acid derivative on the HepG2 human hepatocellular carcinoma cell line. Oncology letters. 2014 Feb; 7: 525-30.
14. Zhang R, Xue YY, Lu SD, Wang Y, Zhang LM, Huang YL, Signore AP, Chen J and Sun FY. Bcl-2 enhances neurogenesis and inhibits apoptosis of newborn neurons in adult rat brain following a transient middle cerebral artery occlusion. Neurobiol Dis. 2006 Nov; 24: 345-56.
15. Karlnoski R, Wilcock D, Dickey C, Ronan V, Gordon MN, Zhang W, Morgan D and Taglialatela G. Up-regulation of Bcl-2 in APP transgenic mice is associated with neuroprotection. Neurobiol Dis. 2007 Jan; 25: 179-88.
16. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S and Boyd MR.New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 1990 Jul; 82(13):1107-12.
17. van Meerloo J, Kaspers GJ,Cloos J. Cell sensitivity assays: the MTT assay. Methods Mol Biol. 2011 Jan; 731:237-45.
18. Ahmed HH, Abd-Rabou AA, Hassan AZ and Kotob SE. Phytochemical Analysis and Anti-cancer Investigation of Bswellia serrata Bioactive Constituents In Vitro. Asian Pacific journal of cancer prevention: APJCP. 2015 Jun; 16 (16): 7179-7188.
19. Esteves M, Siquet C, Gaspar A, Rio V, Sousa JB, Reis S, Marques MPM and Borges F. Antioxidant versus Cytotoxic Properties of Hydroxycinnamic Acid Derivatives – A New Paradigm in Phenolic Research. Arch. Pharm. Chem. Life. Sci. 2008 Mar; 341: 164–73.
20. Chen HM, Wu YC, Chia YC, Chang FR, Hsu HK, Hsieh YC, Chen CC and Yuan SS. Gallic acid, a major component of Toonasinensis leaf extracts, contains a ROS-mediated anti-cancer activity in human prostate cancer cells. Cancer Lett. 2009 Jul; 286: 161–71.
21. Khaledi H, Alhadi AA, Yehye WA, Ali HM, Abdulla MA and Hassandarvish P. Antioxidant, Cytotoxic Activities, and Structure–Activity Relationship of Gallic Acid-based Indole Derivatives. Arch. Pharm. Chem. Life Sci. 2011 Nov; 344: 703–9.
22. Deepa M, Sureshkumar T, Satheeshkumar PK and Priya S. Antioxidant rich Morusalba leaf extract induces apoptosis in human colon and breast cancer cells by the downregulation of nitric oxide produced by inducible nitric oxide synthase. Nutr Cancer. 2013 Feb; 65(2):305-10.
23. Forester SC, Choy YY, Waterhouse AL and Oteiza PI. The anthocyanin metabolites gallic acid, 3-O-methylgallic acid, and 2,4,6-trihydroxybenzaldehyde decrease human colon cancer cell viability by regulating pro-oncogenic signals. Mol. Carcinog. 2014 Jun; 53(6): 432-9
24. Luo H, Jiang BH, King SM and Chen YC. Inhibition of cell growth and VEGF expression in ovarian cancer cells by flavonoids. Nutr Cancer. 2008 Feb; 60: 800-9.
25. Shan BE, Wang MX and Li RQ. Quercetin inhibits human SW480 colon cancer growth in association with inhibition of cyclinD(1) and surviving expression through Wnt/beta-catenin signalling pathway. Cancer Invest. 2009 Jul; 27: 604-12.
26. Alía M, Mateos R, Ramos S, Lecumberri E, Bravo Land Goya L. Influence of quercetin and rutin on growth and antioxidant defense system of a human hepatoma cell line (HepG2). Eur J Nutr. 2006 Feb; 45: 19–28.
27. Birt DF, Hendrich S and Wang W. Dietary agents in cancer prevention: flavonoids and isoflavonoids. PharmacolTher. 2001 May-Jun; 90(2-3):157-77.
28. Nichenametla SN, Taruscio TG, Barney DL and Exon JH. A review of the effects and mechanisms of polyphenolics in cancer. Crit Rev Food Sci Nutr. 2006 Feb; 46(2):161-83.
29. Tian Z, Lin G, Zheng RX, Huang F, Yang MS and Xiao PG. Anti-hepatoma activity and mechanism of ursolic acid and its derivatives isolated from Aralia decaisneana. World Journal of Gastroenterology. 2006 Feb; 12(6):874-9.
30. Murakami A, Ashida H and Terao J. Multitargeted cancer prevention by quercetin. Cancer Lett. 2008 Oct; 269(2):315–25.
31. Lee YK and Park OJ. Regulation of mutual inhibitory activities between AMPK and Akt with quercetin in MCF-7 breast cancer cells. Oncol Rep. 2010 Dec; 24(6):1493–7.
32. Kim HJ, Kim SK, Kim BS, Lee SH, Park YS, Park BK, Kim SJ, Kim J, Choi C, Kim JS, Cho SD, Jung JW, Roh KH, Kang KS and Jung JY. Apoptotic effect of quercetin on HT-29 colon cancer cells via the AMPK signaling pathway. J Agric Food Chem. 2010 Aug; 58(15):8643–50.
33. Wang W and Guan KL. AMP-activated protein kinase and cancer. Acta Physiol (Oxford, England). 2009 Feb; 196(1):55–63.
34. Nakanishi C and Toi M. Nuclear factor NF-κB inhibitors, as sensitizers to anti-cancer drugs. Nat Rev Cancer. 2005 Apr; 5: 297- 309.

35. Lugli E, Ferraresi R, Roat E, Troiano L, Pinti M, Nasi M, Nemes E, Bertoncelli L, Gibellini L, Salomoni P, Cooper EL and Cossarizza, A. Quercetin inhibits lymphocyte activation and proliferation without inducing apoptosis in peripheral mononuclear cells. Leuk Res. 2009 Jan; 33: 140-50.
36. Maas JL and Galletta GJ. Ellagic acid, an anticarcinogen in fruits, especially in strawberries: a review. Hortscience. 1991 Jan; 26: 10-4.
37. Thresiamma KC, George J, Kuttan R. Protective effect of curcumin, ellagic acid and bixin on radiation induced genotoxicity. J Exp Clin Cancer Res.1998 Dec; 17(4):431-4.
38. Lanvin O, Gouilleux F, Mullié C, Mazière C, Fuentes V, Bissac E, Dantin F, Mazière JC, Régnier A, Lassoued K and Gouilleux-Gruart V. Interleukin-7 induces apoptosis of 697 pre-B cells expressing dominant-negative forms of STAT5: evidence for caspase-dependent and -independent mechanisms. Oncogene. 2004 Mar; 23: 3040-7.
39. Dassé E, Bridoux L, Baranek T, Lambert E, Salesse S, Sowa ML, Martiny L, Trentesaux C and Petitfrère E.Tissue inhibitor of metalloproteinase-1 promotes hematopoietic differentiation via caspase-3 upstream the MEKK1/MEK6/p38alpha pathway. Leukemia. 2007 Apr; 21: 595-603.
40. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG and Heber D. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. Journal of Nutritional Biochemistry. 2005 Jan; 1-28.
41. Khanduja KL, Gandhi RK, Pathania V and Syanl N. Prevention of N5 nitrosodiethylamine-induced lung tumorigenesis by ellagic acid and quercetin in 6 mice. Food Chem Toxicol. 1999 Apr; 37: 313-8.
42. Mertens-Talcott SU, Talcott ST and Percival SS. Low concentrations of quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis 9 in MOLT-4 human leukemia cells. J Nutr 2003 Aug; 133: 2669-74.
43. Panaretakis T, Laane E, Pokrovskaja K, Bjorklund AC, Moustakas A, Zhivotovsky B, Heyman M, Shoshan MC and Grander D. Doxorubicin requires the sequential activation of caspase-2, protein kinase Cdelta, and c-Jun NH2-terminal kinase to induce apoptosis. Mol. Biol. Cell. 2005 May; 16(8), 3821-31.
44. Kim SJ; Park KM, Kim N and Yeom YI. Doxorubicin prevents endoplasmic reticulum stress-induced apoptosis. Biochem Biophys Res Commun. 2006 Jan; 339(2): 463-8.
45. Ley R, Ewings KE, Hadfield K and Cook SJ. Regulatory phosphorylation of Bim: sorting out the ERK from the JNK. Cell Death Differ 2005 Aug; 12(8): 1008-14.
46. Zheng Z, Cheng K, Chao J, Wu J and Wang M.Tyrosinase inhibitors from Paper Mulberry (Broussonetia Papyrifera). Food Chemistry. 2008 Jan; 106: 529-35.