An Ethanol Extract of Hawaiian Turmeric: Extensive In Vitro Anticancer Activity Against Human Colon Cancer Cells

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REVIEW ARTICLE

An Ethanol Extract of Hawaiian Turmeric: Extensive In Vitro Anticancer Activity Against Human Colon Cancer Cells

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ABSTRACT

Context • Turmeric (*Curcuma longa*) is a food spice and colorant reported to be beneficial for human health. Curcumin (diferuloylmethane) is the major ingredient in turmeric, and existing data suggest that the spice, in combination with chemotherapy, provides a superior strategy for treatment of gastrointestinal cancer. However, despite its significant effects, curcumin suffers from poor bioavailability, due to poor absorption in the body.

Objective • The research team intended to evaluate a liquid extract of turmeric roots (TEx) that the team had formulated for its in vitro, anticancer activity against several human, colorectal cancer cell lines.

Design • The research team performed in vitro studies evaluating the anticancer efficacy via short and long-term assays and also evaluated invasion using Matrigel (Corning Life Sciences, Tewksbury, MA, USA). Further, in vitro anticancer activity of TEx was tested against 3-D cultures of HCT166 spheroids, which were subsequently analyzed by flow cytometry.

Setting • ADNA, Inc, Columbus, OH, USA; Foundation for Biomedical Research of the Academy of Athens, Athens, Greece; and Laboratory of Pharmacology, Faculty of Medicine, University of Thessaly, Larissa, Greece.

Intervention • The study used 4 human cell lines of colorectal cancer—HT29, HCT15, DLD1, and HCT116—and 2 breast cancer cell lines—SW480 and MDA-MB231. For a short-term assay, the extract was dissolved into culture mediums of HT29, HCT15, DLD1, HCT116, and SW480 at four 10-fold dilutions (100 to 0.1 μg/mL). For a long-term assay, TEx was added to the cultures of the same cell lines at 3 dilutions—20, 10, and 5 μg/mL. For an invasion assay, 100 μL per well of Matrigel was added and allowed to polymerize prior seeding of the MDA-MB231 cells. For cultures treated with the TEx, the TEx was mixed with the cell suspension prior to the seeding step. For the spheroid testing, the TEx was added to HCT116 cells either at the beginning of an experiment (ie, before the addition of the cancer cells), which was a chemopreventive approach, or 48 h later, on the addition of cells to the wells to allow the generation of spheroids, which was a chemotherapeutic approach.

Outcome Measures • The in vitro activities of TEx were evaluated using a 48-h-incubation, short-term assay and a 2-wk, long-term (clonogenic) assay. To analyze the anti-invasive activity of the extract, images for the Matrigel invasion assay were taken with a camera at the 24-h time point. The in vitro, anticancer activity of TEx was also tested against 3-D cultures of HCT116 spheroids that were subsequently analyzed using flow cytometry.

Results • TEx had potently inhibited the growth of all human colon cancer cell lines tested in a dose- and time-dependent manner. TEx inhibited the formation of HCT116 spheroids when the cells were incubated with the extract. The extract also disrupted the formation of tubules formed by MDA-MB231 cells grown on Matrigel at concentrations that did not affect the overall viability of the cells, indicating a potent anti-invasive activity.

Conclusions • These data suggest a potential therapeutic activity for TEx against human colon cancer, most likely due to the enhanced bioavailability of the turmeric. (*Altern Ther Health Med.* 2015;21(suppl 2):46-54.)

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Turmeric (*Curcuma longa*) is a food spice and colorant widely used throughout India and Southeast Asia. Curcumin (diferuloylmethane), the major ingredient in turmeric, is a major constituent of curry powder, to which it imparts its characteristic yellow color. For more than 4000 years, curcumin has been used in traditional Asian and African medicine to treat a wide variety of ailments.[1]

Experimental research has shown curcumin to be a highly pleiotropic molecule capable of interacting with numerous molecular targets in the cell that are involved in inflammation. Based on early cell culture and animal research, clinical trials have indicated that curcumin may have potential as a therapeutic agent iin diseases such as inflammatory bowel disease, pancreatitis, arthritis, and chronic anterior uveitis as well as certain types of cancer.[2]

Curcumin has been shown to inhibit the growth of transformed cells and colon carcinogenesis at the initiation, promotion, and progression stages in carcinogen-induced rodent models. Existing data suggest that curcumin, in combination with chemotherapy, is a superior strategy for treatment of gastrointestinal cancer, with particular reference to colorectal cancer.[3-5] Further, curcumin exhibits antiproliferative effects on human breast cancer cells.[6,7] However, despite these significant effects, curcumin suffers from poor bioavailability, due to poor absorption in the body.[8-12]

Cells cultured as monolayers typically exhibit less resistance to therapy than those grown in vivo. The spheroid generation assay is an excellent model to simulate the development and microenvironmental conditions of in vivo tumor growth. It is assumed that spheroids mimic the tumor (ie, the cells grow in a manner similar to xenografted tumors).[13]

In this study, the research team extracted turmeric from turmeric roots using ethanol. The extract was tested for activity against the various cells of human, colorectal cancer. The team also determined the activity of the TEx on HCT116 spheroid generation and an invasiveness Matrigel (Corning Life Sciences, Tewksbury, MA, USA) model.

METHODS

Procedures

Reagents. All chemicals and cell culture reagents were purchased from Sigma-Aldrich (St Louis, MO, USA), unless otherwise stated. Matrigel biomatrix was purchased from BD Biosciences (Becton Dickinson Hellas, Athens, Greece).

Cell Cultures. The 4 human cell lines of colorectal cancer—HT29, HCT15, DLD1, and HCT116—were obtained from the National Cancer Institute at the National Institutes of Health (Bethesda, MD, USA), whereas the 2 breast cancer cell lines—SW480 and MDA-MB231—were obtained from the American Type Culture Collection (Manassas, VA, USA). All cells were adapted to propagate in RPMI-1640 medium containing 5% heat-inactivated, fetal bovine serum (FBS); 2 mM of L-glutamine; and 1% penicillin-streptomycin. The cell cultures were grown in a 5% CO_2 atmosphere of a humidified incubator at 37°C.

Turmeric Extract. Turmeric roots were obtained from the Hawaiian Organic Farm Association (Hilo, HI, USA). The roots were boiled in water to remove any arsenic contamination and blended in 100% ethanol at a ratio of 1:1 w/v. The turmeric was incubated at room temperature for 2 weeks in glass jugs wrapped in aluminum foil. At the end of the incubation period, the turmeric was filtered through cheesecloth. The residue was stored and tested for presence of arsenic at a later time. The liquid portion was allowed to settle for 24 hours. The supernatant portion was collected carefully without disturbing the bottom layer. The collected portion is hereafter referred to as the *turmeric extract* (TEx). Samples of the extract were also subjected to high-performance liquid chromatography analysis and tested for the presence of arsenic. To concentrate the TEx, the alcohol was allowed to evaporate overnight in a rotator. The residue was suspended in dimethyl sulfoxide (DMSO) at a stock concentration of 20 mg/mL and kept at -20° C for further tests.

In Vitro Studies Against Cancer Cell Lines. After the evaporation of the alcohol, as described earlier, and the resuspension of the residue in DMSO, the in vitro activities of TEx were evaluated using a 48-hour-incubation, short-term assay and a 2-week, long-term (clonogenic) assay. The latter evaluates the ability of a single cell not only to survive, as in the case of the short-term assay, but also to grow into a colony. The 4 colorectal cell lines—HT29, HCT116, HCT15, and DLD1—and 1 breast cancer cell line—SW480—were

used for both the short- and the long-term assays. Cell viability was assessed at the beginning of each experiment by the trypan blue-dye exclusion method and was always greater than 95%.

For the short-term assay, cells were seeded into 96-well cell culture plates in 100 µL of medium at a density of 5000 to 10 000 cells per well. Subsequently the plates were incubated at standard conditions for 24 hours to allow the cells to resume exponential growth prior to addition of TEx. Then, to measure the starting cell population, cells in one plate were fixed in situ with trichloroacetic acid (TCA), followed by sulforhodamine B (SRB) staining, as described elsewhere.[14,15]

To determine the activity of TEx, the extract was dissolved into the culture medium at four 10-fold dilutions—from 100 to 0.1 µg/mL—to cell culture plates, and incubation continued for an additional period of 48 hours. TEx solvent alone (ie, DMSO-containing cell cultures at a maximum final concentration of 0.1% v/v) was used as a negative control. That DMSO concentration corresponded to the highest level that was used in the current study's experimental set-up. DMSO was found to be inert at that concentration (data not shown). The assay was terminated by addition of cold TCA, followed by SRB staining and absorbance measurement at 530 nM in an EL-311 BIOTEK microelisa reader (BioTek, Winooski, VT, USA). Identifying the absorbance measurements as time 0 = Tz, control growth = C, and test growth in the presence of the drug at the different concentration levels = Ti, the percentage growth was calculated at each of the drug concentrations as follows: (1) for concentrations for which $Ti \geq Tz$, $([Ti - Tz] / [C - Tz]) \times 100$; and (2) for concentrations for which $Ti < Tz$, $([Ti - Tz] / Tz) \times 100$.

Three dose-response parameters were calculated. Growth inhibition of 50% (GI50) was calculated from $([Ti - Tz] / [C - Tz]) \times 100 = 50$ (ie, the calculation provided a concentration resulting in a 50% reduction in the net protein increase as measured by SRB staining in control cells during drug treatment). The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The lethal concentration 50 (LC50) (ie, the concentration of the drug resulting in a 50% reduction in the measured protein at the end of the treatment as compared with that of the beginning) indicates a net loss of cells following treatment and was calculated from $([Ti - Tz] / Tz) \times 100 = -50$.[15]

For the long-treatment/clonogenic assay,[16] cells from the same cells lines as were used in the short-term assay were counted, added to 6-well culture plates at a concentration of 50 cells per well, and left for an additional 24-hour adaptation period. After the adaptation period, the TEx was added to the cultures at 3 dilutions—20, 10, and 5 µg/mL. Cells were further incubated for an additional 2-week period. At the end of that period, the experiment was terminated by adding TCA as for the short-term assay. Then cell colonies were photographed and further stained with SRB. Finally, the absorbance was measured by extracting SRB with nonbuffered Tris 10 mM as described earlier. A total of 300 µL were further removed from the 6-well plate to a 96-well microtiter plate, and the absorbance was measured at 540 nM using the EL-311 BIOTEK microplate reader. Inhibiting concentration 50 (IC50) (ie, the concentration that reduces by 50% the optical density generated in the treated wells as compared to the untreated) was further calculated as the percentage of the absorbance of the treated versus untreated cells.

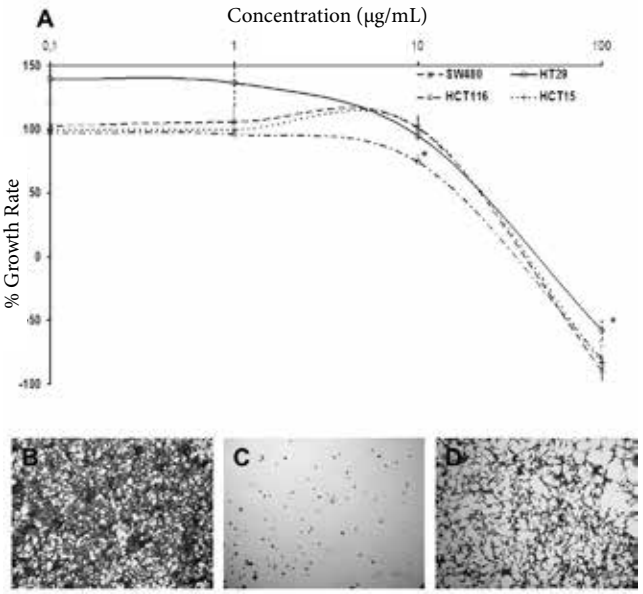
Matrigel Invasion Assay. The anti-invasive activity of the extract was assessed in MDA-MB231 cells grown in Matrigel as previously described,[17] modified for use in 96-well, tissue culture plates. Briefly, 100 µL per well of Matrigel was carefully added to 96-well plates and allowed to polymerize at 37°C for 30 minutes. MDA-MB231 cells were seeded at 20 000 cells per well and allowed to adhere overnight. For wells that were treated with TEx, the TEx was mixed with the cell suspension prior to the seeding step. Images were taken at the 24-hour time point using an inverted microscope equipped with a camera (Zeiss Axiovert 200, Zeiss, Germany). An SRB assay was performed in parallel to confirm that the concentrations used were not cytotoxic for the cells under the experimental conditions.

Generation of Spheroids. Subconfluent, HCT116 cell cultures were treated with trypsin, and the detached cells were counted and seeded into specialized 96-well plates (NanoCulture Plate, Scivax Corporation, Kawasaki, Japan) at a seeding density of 5000 cells per well, following the manufacturers' instructions. Briefly, before plating, cells were suspended in the specialized medium that was provided by the manufacturer with the culture plates. According to the manufacturer, this medium (NCM medium) is a set of basal mediums with antibiotic added and FBS. FBS was lot checked for spheroid formation on the nanoculture plates (ie, the microplates used for the seeding of the cells). Prior to performing the experiment, several cell densities were tested to select the most appropriate for the formation of spheroids. According to the manufacturer, cells seeded into these specific microplates attach to an ultrafine, processed mesh structure on the culture surface, in a spherical morphology. Cells then migrate on the microstructure and continuously contact/adhere to each other and form multicellular clusters, spheroids that grow larger with proliferation of the cells at the same time.

TEx was added to the cells either at the beginning of the experiment (ie, before the addition of the cells), which was the chemopreventive approach, or 48 hours later on the addition of cells to the wells to allow the generation of spheroids, which was the chemotherapeutic approach. Cells were further incubated at 37°C for up to 240 hours (5 d), and images were taken using an inverted microscope equipped with a camera.

Flow Cytometry. To monitor perturbations in the cell cycle and induction of apoptosis, identical cultures of HCT116 cells in spheroids were left untreated or treated, as described earlier. The spheroids were subsequently

Figure 1. Short-term proliferation assay. Treatment of cells with 100 µg/mL of TEx killed the cells.



Abbreviation: TEx, ethanolic extract of turmeric.

Table 1. In Vitro Activity of TEx in the Short-Term Assay[a]

Cell Line	Parameters	Concentration (µg/mL)
HT29	GI50	36.6
	TGI	66.0
	LC50	95.4
HCT116	GI50	24.3[b]
	TGI	53.5
	LC50	82.8
HCT15	GI50	35.2
	TGI	59.5
	LC50	83.8
DLD1	GI50	>100
	TGI	>100
	LC50	>100
SW480	GI50	34.1
	TGI	59.2
	LC50	84.3

Abbreviations: TEx, ethanolic extract of turmeric; GI50, the concentration that inhibits growth of 50% of the cells; TGI, the concentration that completely arrests the growth of the cells; LC50, the concentration that kills 50% of the cells; SRB, sulforhodamine B; CV, coefficient of variation.

[a]The table shows GI50, TGI, and LC50 for the HT29, HCT116, HCT15, DLD1, and SW480 cell lines, as calculated in the short-term assay using the SRB method, CV < 10%.
[b]Denotes $P = .05$.

dissociated using the dissociation solution provided by the manufacturer, treated with ribonuclease (RNase) and stained with propidium iodide immediately before analysis. Subsequently, the cells were analyzed by flow cytometry with the aid of an FC500 flow cytometer equipped with a 488-laser (blue-laser) source (Beckman Coulter, Inc, Nyon, Switzerland).[15]

Statistical Analysis

All analyses were performed with the aid of Prism 5.0 software (GraphPad Software, San Diego, CA, USA). Statistical analysis was performed using unpaired student tests. Differences were considered significant when $P < .05$.

RESULTS

Effect on Cell Proliferation

The antiproliferative and cytotoxic activity of the TEx on various human colorectal cells was determined using short-term proliferation and long-term clonogenic assays. For the short-term assay, the cells were treated with four 10-fold dilution concentrations—100, 10, 1 and 0, 1 µg/mL—of TEx for 48 hours at 37°C. Treatment of cells with 100 µg/mL of TEx killed the cells (Figure 1), with the exception of the DLD1 cells, which were resistant at all concentrations of TEx tested. Concentrations that were below 10 µg/mL had no effect on the growth of the cells.

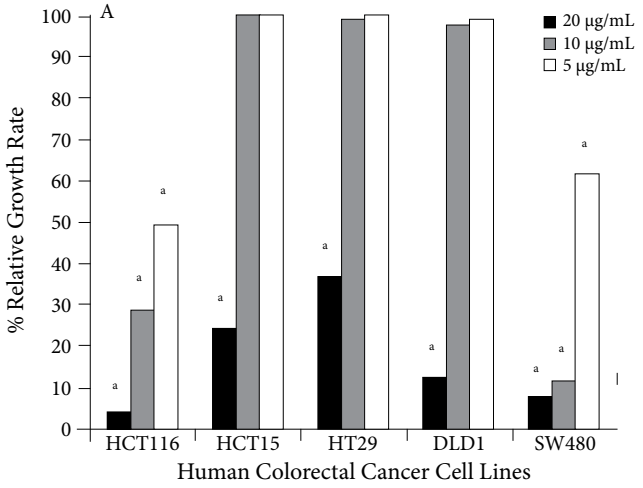
The research team next calculated the following parameters: (1) the GI50 (ie, the concentration that would inhibit growth of 50% of the cells); (2) the TGI (ie, the concentration that would completely arrest the growth of the cells); and (3) the LC50 (ie, the concentration that would kill 50% of the cells) (Table 1). Among all cell lines tested, HCT116 cells were the most sensitive, as depicted by the corresponding GI50, which was calculated to be 24.3 µg/mL (Table 1).

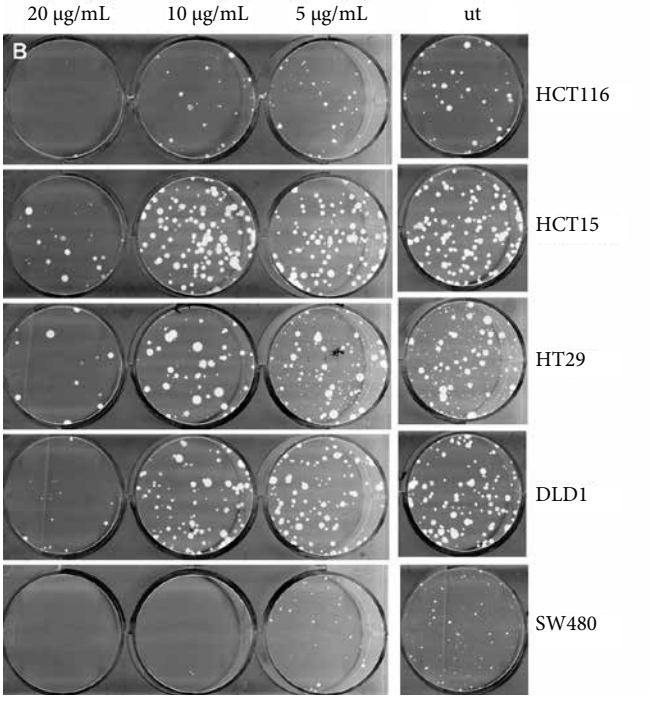
To test in a more extended in vivo–related way, the research team subsequently performed a long-term, modified clonogenic assay (Figure 2; Table 2). In the clonogenic assay, the TEx was more potent than in the short-term assay. TEx, at concentrations as high as 20 µg/mL, could inhibit proliferation of all cell lines, including the DLD1 cells, which were shown to be the most resistant in the short-term, 48-hour assay. The HCT116 cells were the most sensitive to the TEx treatment, with an IC50 at 4.3 µg/mL, followed by the SW480 cells at IC50 at 7.1 µg/mL (Table 2).

Anti-invasive Activity in Matrigel

To evaluate the anti-invasive potential of the TEx, MDA-MB231 cells that are able to form extensive tube networks under normal conditions[17] (Figure 3A) were treated with noncytotoxic concentrations (50 and 20 µg/mL) of TEx for 16 hours (Figure 3D). As can be seen in Figures 3B and 3C, both concentrations of TEx were highly active in preventing tubule formation in MDA-MB231 cells, suggesting a potent anti-invasive activity for the extract.

Figure 2. Long-term, modified clonogenic assay. TEx was more potent than in the short-term proliferation assay.

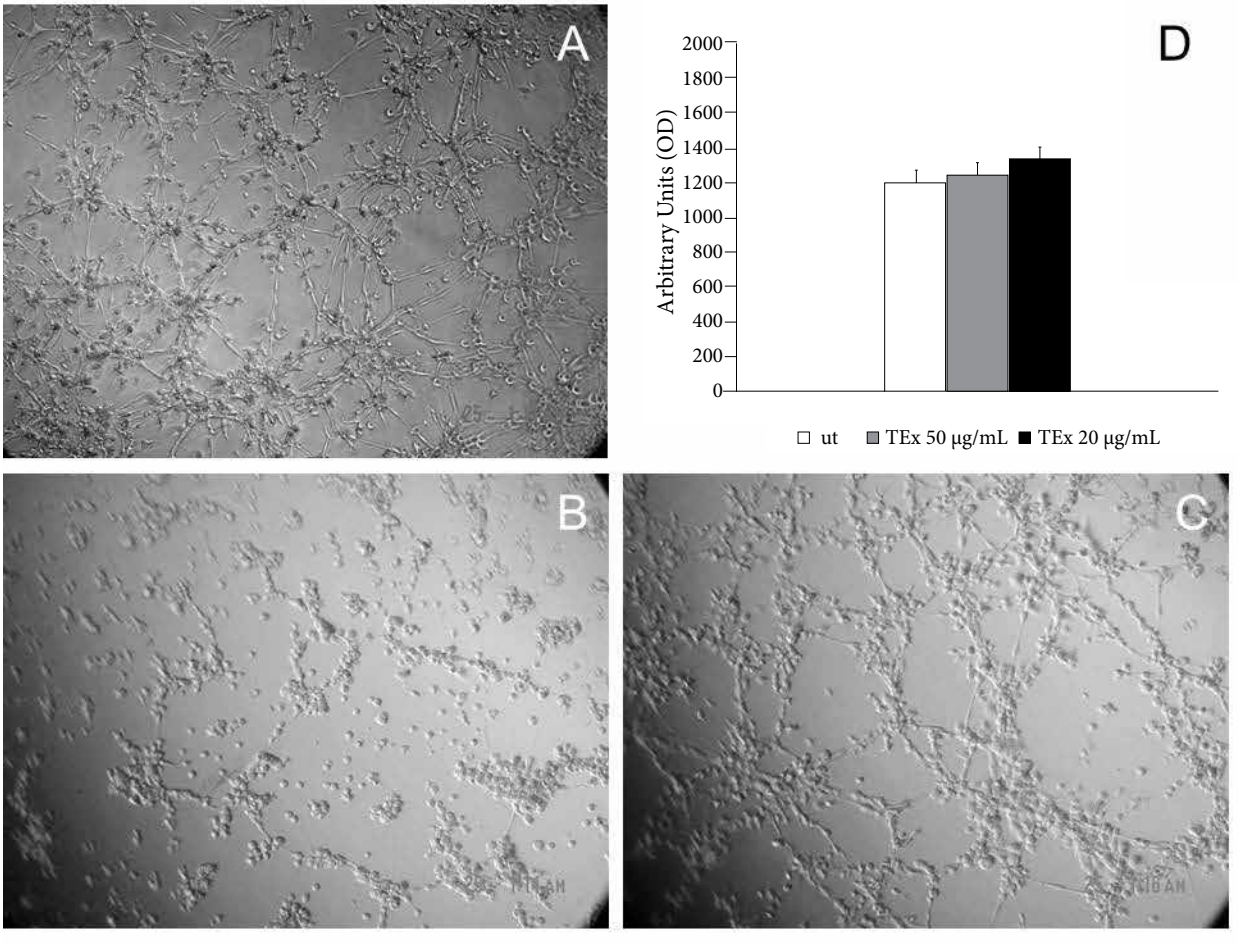




Abbreviations: TEx, ethanolic extract of turmeric; ut, untreated cells.

[a]Denotes P =.01 compared with the corresponding control.

Figure 3. Anti-invasive activity in Matrigel.



Abbreviations: OD, optical density; ut, untreated cells; TEx, ethanolic extract of turmeric.

Figure 4. Effect on the formulation of HCT116 spheroids: preincubation.

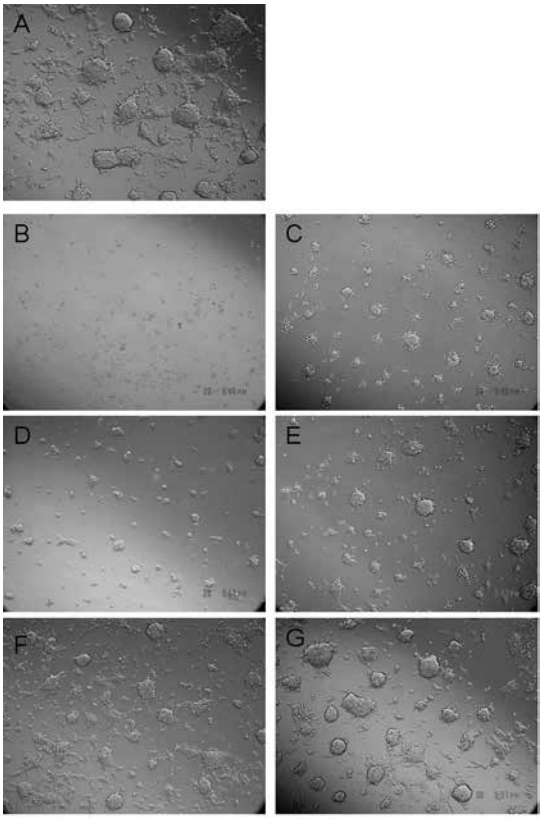


Table 2. In Vitro Activity of the TEx in the Long-term/ Clonogenic Assay[a]

Cell Lines	IC50 (µg/mL)
HCT116	4.3
HCT15	15.8
HT29	17.4
DLD1	14.6
SW480	7.1

Abbreviations: TEx, ethanolic extract of turmeric; IC50, inhibiting concentration 50; CV, coefficient of variation.

[a]The table shows the IC50 values of TEx for the HCT116, HCT15, HT29, DLD-1, and SW480 cell lines, as calculated by the long-term/clonogenic assay, CV < 15%.

Figure 5. Effect on the formulation of HCT116 spheroids: postincubation.

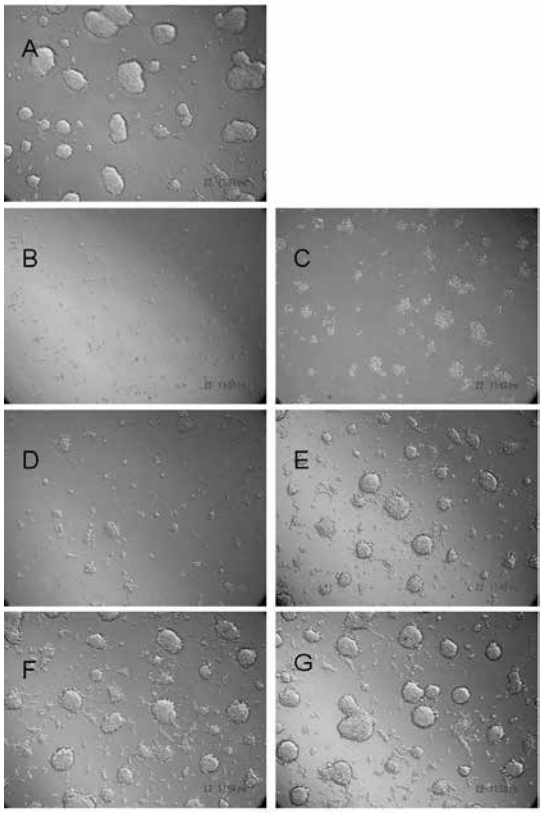
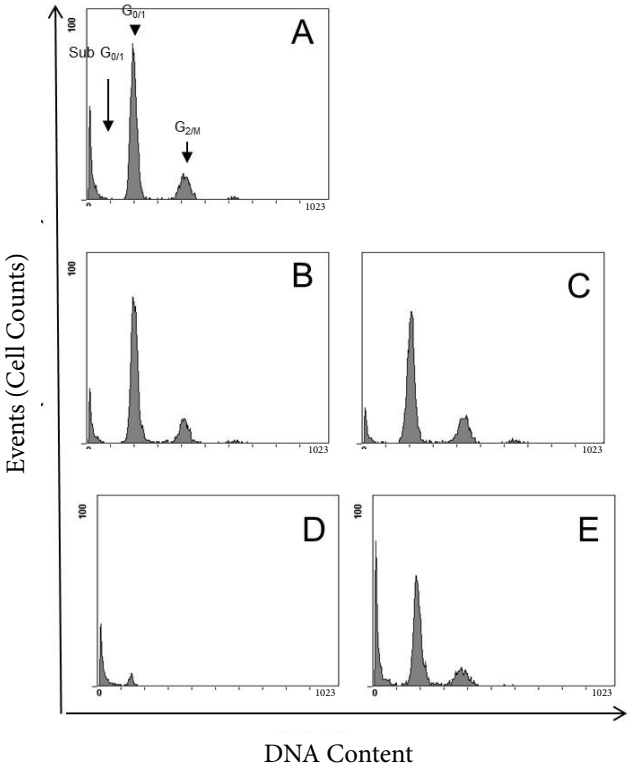


Figure 6. Cell count and DNA content.



Effect on the Formulation of HCT116 Spheroids

The spheroid generation assay is a useful tool to simulate the in vivo environment under in vitro conditions. It is assumed that spheroids mimic a tumor (ie, the cells grow in a manner similar to xenografted tumors).[13] In the current study, HCT116 cells were incubated on a special culture plate to initiate generation of spheroids. HCT116 cells were selected as they were found to be the most sensitive to TEx activity. The cells were either preincubated, the chemopreventive assay, or coincubated after the formation of spheroids, the chemotherapeutic assay, with various concentrations of TEx—100, 50, 20, and 10 µg/mL. As is demonstrated in Figure 4, TEx was able to abolish the formation of HCT116 spheroids.

When cells were incubated with 100 µg/ml of the TEx, no spheroids were formed regardless of pre- or postincubation (Figures 4B, 4C, 5B, and 5C). Fifty µg/mL of TEx inhibited the formation of spheroids; however at that concentration, TEx was more effective when cells were preincubated with the same concentration (Figures 4D, 4E, 5D, and 5E). Finally, when cells were pretreated with 20 µg/mL, the spheroids that were formed seemed to be smaller in size as compared with the untreated cells at 48 hours postspheroid formation (Figure 4F), but the effect was ultimately abolished. No effect was observed in the presence of the 10-µg/mL concentration.

Finally, the current research team performed a flow-cytometry analysis to determine whether the cells were propagating and still alive after 144 hours in culture, with only 1 medium change. The team was also interested in determining whether any effect was seen on the cell cycle and type of death induced on TEx treatment. The team observed that the cells grew well, although a relatively high percentage of sub-$G_{0/1}$ cells, indicative of apoptotic cells, were observed (Figure 6A). Because of the extreme culture conditions, the death percentage for untreated and solvent-treated control cells was expected to be high, similar to the spontaneous necrosis observed in HCT116 and other rapidly growing tumors in xenografts.[15] All cells treated with 100 µg/mL were dead and, therefore, could not be further analyzed by flow cytometry (Table 3).

A sub-$G_{0/1}$ population was apparent in 50 µg/mL pre- and posttreated spheroids, but an important difference existed between post- and pretreated cells (Figures 6D and 6E and Table 3). No effect on the cell-cycle phase distribution was observed. Finally, no differences were observed in cells treated with 20 µg/mL or 10 µg/mL. The results suggest that the TEx exhibited a significant inhibitory activity on spheroid formation, which was time and dose dependent, and that TEx can function in a chemopreventive as well chemotherapeutic manner. However, it appears that because of the time-dependent effect of the extract TEx, it may be more potent in a chemopreventive manner.

Table 3. FACS Analysis of HCT116 Cells Isolated From Spheroids[a]

	Sub-$G_{0/1}$	$G_{0/1}$	S	$G_{2/M}$
ut	19	73.1	5.8	21.1
10 post	14, 2	73.1	7.6	19.3
10 pre	9, 46	71.3	8.1	20.6
20 post	9, 02	67.9	8.7	23.4
20 pre	11, 84	69.5	9.7	20.8
50 post	26, 08	62.1	14.1	23.8
50 pre	85, 3	ND	ND	ND
100 post	86, 2	ND	ND	ND
100 pre	69, 8	ND	ND	ND

Abbreviations: FACS, fluorescence-activated cell sorting; CV, coefficient of variation; ut, untreated cells; ND, not d-performed due to the high percentage of dead cells.

[a]Analysis of the cells in various phases of the cell cycle was performed only with live cells. Results are the mean of at least 2 independent experiments, CV ≤ 12%.

DISCUSSION

Turmeric (*Curcuma longa*) is an herbaceous perennial plant belonging to the botanical family of Zingiberaceae, the ginger family, with the rhizome being the most valued plant part, used for cooking and medicine.

Various forms of turmeric extracts have been reported to show beneficial properties for human health. Turmeric extract has been shown to (1) exhibit antioxidant activity, protecting rabbits from atherosclerosis[18,19]; (2) offer protection from irradiation in mouse models[20,21]; (3) induce apoptosis of Ehlrich ascitic carcinoma in mice[22]; and (4) act in a chemopreventive manner, protecting from cancer.[23,24] In a partially blinded, randomized, 2-dose pilot study, Bundy et al[25] reported that oral administration of a standardized turmeric extract may help to reduce symptomology for irritable bowel syndrome. However, to the current research team's knowledge, no evaluation has been done on either the chemoprotective or the anticancer activity of any type of turmeric extract with regard to human colon cancer.

In the current study, the research team had prepared an ethanolic extract of turmeric (TEx), which was subsequently tested for antiproliferative and cytototoxic activity on various human colorectal and breast cancer cells, using a short-term proliferation and a long-term clonogenic assay. The results from both assays suggested that prolonged incubation times can greatly improve the activity of the ethanolic extract and that the activity of the TEx is clearly time and dose dependent but also is dependent on the specific colon cancer cell line (Tables 1 and 2).

It has been previously demonstrated that cancer cell lines can form networks on Matrigel and that this property is dependent on the invasiveness of the cells.[17] Accordingly, a simple assay was set up in the current study to identify agents with anti-invasive potential. In that assay, which was

performed using MDA-MB231 cells, TEx was found to prevent tubule formation of those cells, thus suggesting a potent anti-invasive activity for the extract.

Finally, it is assumed that formation of spheroids mimics tumor cell behavior in xenografted tumors.[13] Therefore, HCT116 cells were grown in specialized media to form spheroids, and the effect of TEx on them was determined in the current study. In that assay, the cells were either preincubated or coincubated after the formation of spheroids, a chemopreventive or a chemotherapeutic assay, respectively, with various concentrations of TEx. TEx was able to decrease the formation of HCT116 spheroids and was more effective when cells were preincubated with the extract. This result may demonstrate that TEx could be more potent as a chemopreventive than as a chemotherapeutic agent.

The subsequent fluorescence-activated cell sorting (FACS) analysis of the cells that originated from spheroids revealed a sub-$G_{0/1}$ population consistent with dying cells, most probably apoptotic, but no differences were observed with regard to cell cycle phases. Both the spheroid assay and the FACS analysis revealed once again that the effect of TEx was time and dose dependent.

In this article, the research team has presented evidence to demonstrate a significant in vitro, antiproliferative potential for a liquid extract of Hawaiian turmeric roots. It is well known, and high-performance liquid chromatography (HPLC) analysis has revealed, that curcumin is one of the main ingredients of the extract, and some of the properties could be attributed to the extremely significant biological properties of that phytochemical. It is thus difficult to discuss turmeric separately from its major ingredient, curcumin. Curcumin has been shown in many biological systems to exhibit antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities alone or as an adjunct to overall cancer treatment.[26-37] It is physiologically very well tolerated, demonstrating low systemic toxicity, having been shown to be safe at high doses, with a tolerance of up to 12 g in a single oral dose.[38]

Its applications are limited, however, mainly due to its poor pharmacokinetic properties—low solubility and physicochemical stability, rapid systemic clearance, and low cellular uptake. Nevertheless, such extracts are well enriched in other important phytotherapeutics, including anthocyanins and flavonoids, and this fact is underlined by reports that have shown beneficial effects for curdumin-free or aqueous turmeric extracts.[23,24] Thus studies with isolated constituents are needed to clarify whether the effects are due to a single ingredient or to a combination of constituents, and they are in progress in the research team's laboratories. Future studies will also include the effect of TEx on animal xenografts that will be injected with TEx-treated HCT116 cells, or other types of cancer, in an effort to shed light on the chemopreventive activity of this extract.

CONCLUSIONS

The current study's findings indicate that TEx, an ethanolic extract of turmeric roots, may have potential therapeutic benefits against colon cancer, which is a turmeric-extract property that has been demonstrated, to the research team's knowledge, for the first time in the current study. The team has currently initiated an extensive investigation to optimize the process of extracting TEx at a high concentration. Future studies should address the effects of TEx in mice bearing established human HCT116 tumors as well as other tumors of diverse origin.

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AUTHOR DISCLOSURE STATEMENT

All the authors declare that they had no financial or commercial conflicts of interest related to the current study. The work was partly funded by NIH grant No. 3R44AT004118-03S1.

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