

RESEARCH ARTICLE

Gelam Honey and Ginger Potentiate the Anti Cancer Effect of 5-FU against HCT 116 Colorectal Cancer Cells

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Abstract

The development of chemopreventive approaches using a concoction of phytochemicals is potentially viable for combating many types of cancer including colon carcinogenesis. This study evaluated the anti-proliferative effects of ginger and Gelam honey and its efficacy in enhancing the anti-cancer effects of 5-FU (5-fluorouracil) against a colorectal cancer cell line, HCT 116. Cell viability was measured via MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium) assay showing ginger inhibiting the growth of HCT 116 cells more potently (IC₅₀ of 3mg/mL) in comparison to Gelam honey (IC₅₀ of 75mg/mL). Combined treatment of the two compounds (3mg/mL ginger+75mg/mL Gelam honey) synergistically lowered the IC₅₀ of Gelam honey to 22mg/mL. Combination with 35 mg/mL Gelam honey markedly enhanced 5-FU inhibiting effects on the growth of HCT 116 cells. Subsequent analysis on the induction of cellular apoptosis suggested that individual treatment of ginger and Gelam honey produced higher apoptosis than 5-FU alone. In addition, treatment with the combination of two natural compounds increased the apoptotic rate of HCT 116 cells dose-dependently while treatment of either ginger or Gelam honey combined with 5-FU only showed modest changes. Combination index analysis showed the combination effect of both natural compounds to be synergistic in their inhibitory action against HCT 116 colon cancer cells (CI 0.96 < 1). In conclusion, combined treatment of Gelam honey and ginger extract could potentially enhance the chemotherapeutic effect of 5-FU against colorectal cancer.

Keywords: Gelam honey - ginger - HCT 116 cells - anti-proliferation - apoptosis - 5-FU - colon cancer

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Introduction

Colorectal cancer is considered as one of the major causes of morbidity and mortality in the world. This disease accounts for 9.7% of all cancer deaths, and is the most commonly diagnosed cancer after lung and female breast cancer (Ferlay et al., 2010). In Malaysia, colorectal cancer is the second most frequent type of cancer with high prevalence in male than to female population (Omar et al., 2006).

Contemporary therapeutic methods for this type of cancer have been primarily on surgical measures and chemotherapy (Mayer, 2009). However, despite remarkable progress in these approaches, effective therapeutic outcome is minimal owing to the incomplete eradication of the disease and unfavorable side effects, which regularly impede effective treatment (Braun and Seymour, 2011). 5-Fluorouracil (5-FU) is one of the most commonly used therapeutic agents in first-line therapy against colorectal cancer. The drug exerts its anti-cancer effects by inhibiting the activity of thymidilate synthase

(TS) and disrupting DNA & RNA synthesis (Longley et al., 2003) While its survival benefit has been firmly established, clinical applications of the drug have been greatly restricted due to the acquired drug resistance as well as toxicity in higher dosage (Meregalli et al., 1998). In this sense, new strategies involving several anti-tumor modulators have been applied in combination of this drug to boost its efficacy while conserving acceptable toxicities. The beneficial effect of combined drug treatment such as the anti-cancer drugs Oxaliplatin +5-FU or Irinotecan +5-FU was observed to increase patient's survival rate thus expanding the median survival period (Douillard et al., 2000; Giacchetti et al., 2000).

The concept of dual therapy of anti-cancer drugs with natural compounds has become a very promising approach in new drug discovery. Phytochemicals from natural resources, readily available in our daily diet are now being utilized for chemoprevention (Rajamanickam and Aggarwal, 2008). Thus, the new concept of combined chemoprevention by multiple agents or by the consumption of "whole foods" has become an increasingly attractive

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area of study (Mehta et al., 2010). Several reports have described the ability of these naturally derived compounds to modulate the process of carcinogenesis; either by blocking or reversing, with nominal cytotoxicity (Braun and Seymour, 2011; Lee et al., 2011). For example the use of natural compounds in combination with 5-FU may provide effective colorectal cancer treatment by enhancing tumoricidal activity while reducing dose-related toxicity and resistance.

Ginger (*Zingiber officinale*) is one of many dietary substances that are rich in phytochemicals. For centuries, it has been used as food flavoring agent as well as for traditional medicinal purposes (Ali et al., 2008). Current trend in research has focused its attention to ginger given its anti-cancer, anti-oxidant, and anti-inflammatory properties (Masuda et al., 2004; Grzanna et al., 2005; Shukla and Singh, 2007; Habib et al., 2008). Recently, our laboratory has demonstrated anti-tumor effects of ginger extracts against colon cancer cell lines through the induction of apoptotic pathway coupled with cell cycle arrest at G₀/G₁ phase (Abdullah et al., 2010). Ginger extracts is known to contain numerous potent phytochemicals including gingerols, shogaols, zingerols, and paradols (Jolad et al., 2004). Of these phenolic constituents, shogaols and gingerols are the principal active pharmacological components of ginger. They exhibited growth-inhibitory effects against other cancer cells namely lung, ovarian, and skin cells (Kim et al., 2008).

Honey is a natural product of bees, rich in phenolic compounds and other essential vitamins and enzymes (Cherchi et al., 1994; Havsteen, 2002; Jaganathan and Mandal, 2009). Studies have indicated the range of health benefits of honey, which includes anti-inflammatory, antioxidant and wound healing activities (Kassim et al., 2010; Mohamed et al., 2010; Al-Waili et al., 2011; Hussein et al., 2012). Honey was also known to exert anti-cancer effects in several types of cancer cells including colon cancer (Jaganathan et al., 2010; Fukuda et al., 2011). Malaysian local honey especially Tualang honey has been found to induce anti-proliferative effects on cervical and breast cancer cell lines (Mohamed et al., 2010) as well as anti-bacterial activity against *Acinetobacter baumannii* (Fauzi et al., 2011). Likewise, another local honey, Gelam honey has been shown to inhibit the growth of colon cancer cell line namely HT 29 (Wen et al., 2012).

In this study, the anti-proliferative and apoptotic effects of ginger, Gelam honey and 5-FU on *k-ras* mutated cell line, HCT 116 were determined. The effects of 5-FU with ginger or Gelam honey were then compared to evaluate the ability of both compounds to boost the impact of 5-FU on growth inhibition and on cellular apoptosis.

Materials and Methods

Materials

Zingiber officinale (ginger) water extract was kindly provided by Noor Azian Morad (Professor, Centre for Lipids Engineering Applied Research, Universiti Teknologi Malaysia). Briefly, 75g of dried and ground ginger were weighed before being loaded into a mesh cylinder. The cylinder was then put in a specified reactor

before 700ml of distilled water was added. N₂ gas was then allowed into the reactor to remove remaining O₂ gas. Temperature was subsequently set to 140°C while the pressure at 35 Bar prior extraction process. Once the extraction process is completed, ginger water extract was transferred into another reactor where temperature and pressure were set to 25°C and 10 Bar respectively to allow rapid cooling process to occur. Ginger extracts were then collected and subjected for further analysis. Ginger was freeze-dried before its use in all experiments.

Gelam honey was provided by the National Apiary, Department of Agriculture, Batu Pahat, Johor, Malaysia. The honey was sent to SINAGAMA, Malaysian Nuclear Agency for sterilization using the Cobalt-60 source (Model JS10000). The irradiation process was done at 25kGy and subsequently stored at 4°C for future use. Ginger extract and Gelam honey were diluted with complete culture medium at varying concentrations for each experiment.

5-Fluorouracil (5-FU) was purchased from Sigma (USA). The drug was prepared as 1M stock solution in dimethyl sulfoxide (DMSO) (Sigma, USA) and stored at -20°C. For every experiment, 5-FU was diluted with cell culture medium to the range of concentration indicated at a final DMSO concentration of 0.1% (v/v). All chemicals and reagents consumed were of analytical grade.

Cell culture and treatment

HCT 116 cell line was obtained from the American Type Culture Collection (Rockville, USA) and was cultured in T-25 flask containing McCoy 5A Medium Modified equipped with L-glutamine (Invitrogen, USA) supplemented with 10% fetal calf serum (FCS) (PAA Laboratories GmbH, Austria), 100U/mL Penicillin-Streptomycin (Flowlab, Australia), and 100U/mL Amphotericin B (Flowlab, Australia) at 37°C in a humidified atmosphere of 5% CO₂. Cells were maintained as a monolayer and sub-cultured every 3 days. Cells were used when monolayer reached 70% confluence in all experiments.

Cell viability assay

MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphenyl)-2H-tetrazolium) (Promega, USA) colorimetric assay was used to assess cell viability. Briefly, cells were collected via trypsinization and separated into single-cell suspensions in complete culture medium. Cells were then plated into wells of 96-well plate with the density of 2×10⁴ cells or 1×10⁴ cells (for 5-FU experiments) per 100µL complete medium (McCoy 5A Medium Modified). Cells were then incubated overnight at 37°C in 5% CO₂. Culture medium was then removed before ginger (1-10mg/mL), Gelam honey (10-110mg/mL) and 5-Fluorouracil (10-100µM) (Sigma, USA) were added either individually or combined. Cells were then incubated for another 24h or 72h. After the incubation period, culture medium was discarded and replaced with MTS solution (Promega, USA) (20µL MTS diluted in 100µL complete medium) before being incubated again at 37°C, 5%CO₂ for another 2h. The resulting products were determined spectrophotometrically at the absorbance of 490 nm and 405 nm via enzyme-linked immuno-sorbent

assay (ELISA) microplate reader (VERSAmax, USA). Each experiment was performed in triplicate.

Combined treatment of ginger and honey on HCT 116 cells

Prior combination of ginger and Gelam honey, individual IC_{50} (the dose at which 50% of maximal proliferation was inhibited) was identified from single treatment of HCT 116 cells under the conditions specified above. To determine the optimal combined inhibitory effects, HCT 116 cells were incubated concurrently for 24h with fixed concentration of ginger (2mg/mL) and varying concentrations of Gelam honey (1-30mg/mL).

Combination index (CI) analysis

Drugs combination effect was determined by Chou-Talalay equation (Chou, 2006), $CI = (d_A/D_A) + (d_B/D_B)$, where, d_A and d_B are the doses of individual drugs alone, i.e. concentration of 5-FU or ginger/honey respectively that gives 50% inhibition. DA and DB are the doses of 5-FU and ginger/honey in combination that inhibits 50% of cell growth. $CI > 1$, $CI = 1$ and $CI < 1$ indicate antagonistic, additive and synergistic effect, respectively

Cell apoptosis measurement by ELISA

Mono- and oligonucleosomes in the cytoplasmic fraction were quantified via the Cell Death Detection ELISApplus Assay Kit (Roche Diagnostics, Germany) according to the manufacturer's instruction. Briefly, HCT 116 cells were initially plated in 96-well plates and were allowed to attach overnight. Following overnight incubation period, the cells were then treated with ginger, Gelam honey and 5-Fluorouracil (individually or combined) under the conditions detailed previously. After respective incubation periods, HCT 116 treated cells were centrifuged and the supernatant retained. 20 μ l aliquots of the supernatant were then added into the streptavidin-coated 96 well microliter plates followed by 80 μ l of immunoreagent, which composed of anti-histone antibody and anti-DNA-Peroxidase cocktail. The plate was incubated for 2h at 37°C before individual wells were washed with incubation buffer. 100 μ l of ABTS solution (substrate peroxidase) was then added into respective wells before the amount of nucleosomes were spectrophotometrically determined. Absorbance was measured at 405 nm via the ELISA microplate reader. The results were demonstrated as the ratio of the absorbance of treated cells to the control (untreated cells). The experiment was conducted in duplicate.

Statistical analysis

All data are presented as mean \pm standard deviation (SD). Data were analyzed using Repeated Measure – One-Way Analysis of Variance (ANOVA) followed by Tukey's post-hoc tests, and Repeated Measure - Two-Way ANOVA to determine differences between groups. A p value of < 0.05 was considered statistically significant.

Results

Effects of ginger and Gelam honey on HCT 116 cells

Figure 1A and Figure 1B showed effective growth

inhibitory effects of ginger and Gelam honey on HCT 116 cells in a dose dependent manner. Single treatment with Gelam honey exhibited a gradual decrease in cell viability as the concentration of honey was increased reaching an IC_{50} of 75mg/mL. In contrast, ginger showed much steeper decline with regards to its anti-proliferative ability against HCT 116 cells reaching an IC_{50} of 3mg/mL. This indicates that ginger is much more potent in inhibiting the growth of HCT 116 cells in comparison to Gelam honey.

Effects of combined treatment of ginger extract and gelam honey on HCT 116 cells proliferation

In Figure 1C, 2mg/mL of ginger was shown to synergistically assist Gelam honey in inhibiting the growth of HCT 116 cells reaching an IC_{50} of less than 30mg/mL compared to honey alone (75mg/mL). The Combination Indices (CI) determined showed a $CI = 0.960$ ($CI < 1$) which demonstrates synergism between the two natural compounds.

Effects of ginger and gelam honey co-treatment with 5-FU on HCT 116 cells growth

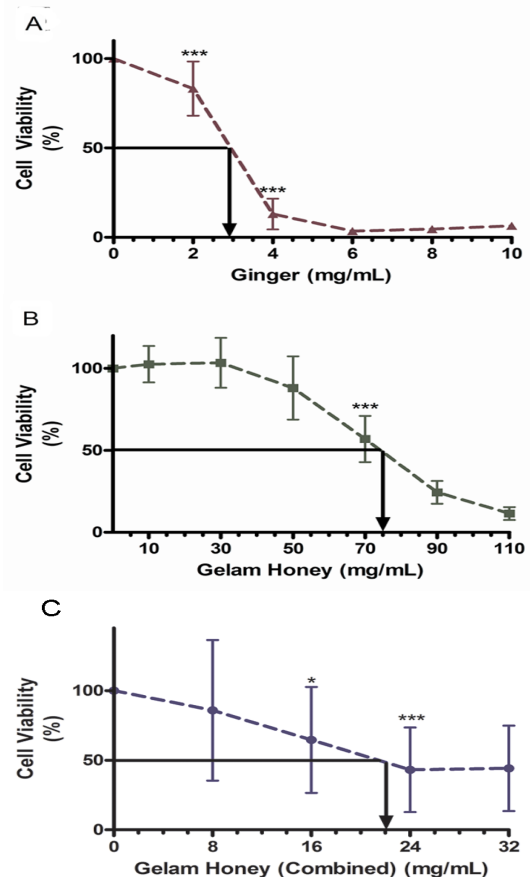


Figure 1. Growth Inhibitory Effects of Ginger (*Zingiber officinale*) and Gelam Honey on HCT 116 Cells.

Following an overnight incubation, HCT 116 colon cancer cells were then treated with either (A) ginger or (B) Gelam honey at varying concentrations and with (C) combination of 2mg/ml of ginger and varying dose of Gelam honey. MTS assay was performed after 24h and percentage of viable cells was measured in triplicate (presented as mean \pm SD, n=3). Statistical analysis was performed using Repeated-Measure ANOVA, followed by Tukey's Post-Hoc test. A p value of < 0.05 was considered statistically significant. ***p < 0.001 vs. control

As illustrated in Figure 2, single and combination treatment of 5-FU with either ginger or Gelam honey exhibited significant reduction in cell growth as compared to the control (untreated) group. 5-FU's ability to affect the growth of HCT 116 cells was further enhanced only when the drug was combined with Gelam Honey. The combination of a single dose 35mg/mL of Gelam honey with 10, 50, 100 μ M of 5-FU displayed significant suppressive effect on HCT 116 growth in contrast to 5-FU alone at the same dosage. The co-treatment effect was markedly 3-fold higher resulting in reduced cell growth by 72%, 75% and 80% of total growth (as compared to 5-FU alone). Ginger on the other hand, exhibited different readings, as co-treatment of ginger (1.75mg/mL) with 5-FU (of 10, 50, 100 μ M) did not show significant increase in the ability of drug to reduce the growth of HCT 116 cells. The trend was found to be similar to the treatment of 5-FU alone. Collectively, these results suggest that 5-FU treatment reduced the growth of HCT 116 cells, but the effect was markedly enhanced when combined with Gelam honey.

Effects of ginger and gelam honey on 5-FU-induced stimulation of apoptosis in HCT 116 cells

As shown in Figure 3A, single Gelam honey treatment resulted in induction of apoptotic activity (in comparison to the control), The value was found to be nearly 5-fold higher to that of the control (untreated) samples. However there was not much difference in apoptotic activity between doses of Gelam honey tested.

For ginger treatment, a different trend was found as illustrated in Figure 3B. 3mg/mL of ginger was found to induce the highest apoptotic activity by 4-fold in comparison to that of the control and 2mg/mL treated samples. The increased apoptotic activity was significant when compared to the control ($p < 0.05$).

However, with regards to the combined treatment of the two natural compounds, only a moderate increase of apoptotic activity was observed in treated samples (Figure

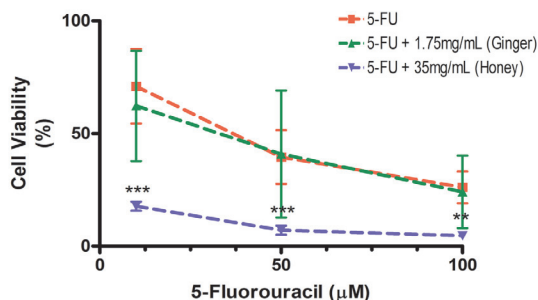


Figure 2. Growth Inhibitory Effects of 5-Fluorouracil Alone and in Combination with Ginger or Gelam Honey on HCT 116 Cells. After an overnight incubation cells were treated with 5-Fluorouracil at (10 μ M, 50 μ M, and 100 μ M) and in combination with fixed concentration of ginger or Gelam honey and MTS assay was performed after 72h. Percentage of viable cells gained from individual and co-treatment of the drug was measured in triplicate (presented as mean \pm SD, n=3). Statistical analysis was performed using Repeated-Measure Two-way ANOVA, followed by Bonferroni post-tests. A p value of <0.05 was considered statistically significant. **p<0.01, ***p<0.001 vs. 5-FU

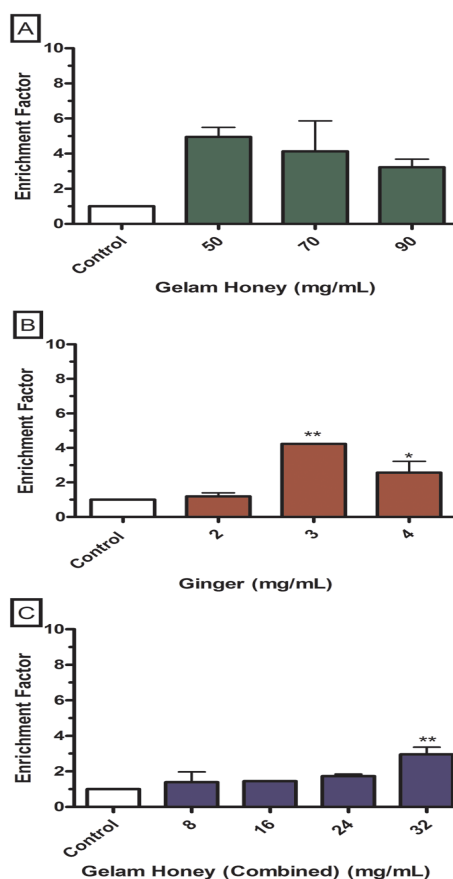


Figure 3. Induction of Apoptosis of HCT 116 Cells by Ginger and Gelam Honey. Cells were treated with (A) Gelam honey, (B) ginger, and (C) combination of both (2mg/mL of Ginger combined with varying concentrations of Gelam honey). After 24 h, nucleosomes in the cytoplasmic fraction from treated cells were quantified via the Cell Death Detection ELISApplus Assay Kit. Apoptotic activity was expressed as enrichment factor [the ratio of the absorbance value of treated cells to the Control (untreated cells)]. Experiments were done in duplicates (presented as mean \pm SD, n=1). Statistical analysis was performed using Repeated-Measure ANOVA, followed by Tukey's Post-Hoc test. A p value of <0.05 was considered statistically significant. *p<0.05, **p<0.01 vs. control

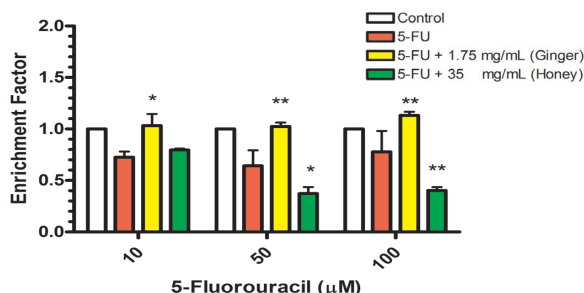


Figure 4. Effects of 5-Fluorouracil Alone and in Combination with Ginger or Gelam Honey on the Induction of Apoptosis in HCT 116 Cells. Cells were treated with 5-Fluorouracil alone at 10 μ M, 50 μ M, and 100 μ M or co-treated with fixed concentration of ginger or Gelam honey (at the same dosage). After 72h, nucleosomes from drug-treated cells were then measured using the Cell Death Detection ELISApplus Assay Kit as described in Figure 3. Statistical analysis was performed using Repeated-Measure Two-way ANOVA, followed by Bonferroni post-tests. A p value of <0.05 was considered statistically significant. *p<0.05, **p<0.01 vs. 5-FU

3C). The combination dose of 32mg/mL of Gelam honey with 2mg/mL of ginger induced significantly higher apoptotic activity in HCT 116 treated cells.

While single and combination treatment of ginger and Gelam honey increased the apoptotic activity, surprisingly single 5-FU treatment (10, 50, 100 μ M) produced in significant changes in apoptotic activity post-treatment (when compared to control). However, combinations of 5-FU treatment with either natural compound, particularly ginger extract caused significant changes in the apoptotic activity (Figure 4). Apoptotic activity of 5-FU combined with 1.75mg/mL ginger significantly increased for all dose combination post-treatment. While this is true for ginger, co-treatment of the drug (at 50 and 100 μ M) with 35mg/mL of Gelam honey surprisingly resulted in significant decrease of apoptotic activity.

Discussion

Combination therapy in the approach of killing cancer cells have been a major focus in the formulation of effective concoction of chemotherapeutic drugs such as 5-FU in combination with oxaliplatin in the treatment of colon cancer. Due to concern on minimizing the adverse side effects from these drugs, concoction of natural products that are often present in our dietary food regime such as ginger and honey could be potential chemopreventive agents against colon adenocarcinoma since they are considered as safe food (Zhu et al., 2013). Consumptions of foods rich in phytochemical antioxidants has been shown to have chemopreventive effect against many types of cancers (Aggarwal et al., 2004). In this present study, crude ginger (*Zingiber officinale*) extract and Gelam honey were tested in combination doses in inhibiting growth of colon cancer cells HCT 116. Inhibiting cell proliferation and inducing apoptosis in cancer cells are effective strategies for combating tumor (Manju and Nalini, 2005).

Our data indicate a dosage of about 3 mg/mL ginger extract is sufficient to inhibit 50% growth (IC_{50}) of HCT 116 colon cancer cells. It is quite interesting to note that the same dose of ginger extract is required for inducing highest apoptotic activity in the cancer cells (Figures 1A and 3B), This could suggest that 3mg/ml is a very effective dose of ginger extract for optimized anti-cancer effect on colon adenocarcinoma. Other studies have also reported the anti proliferative property of ginger extract in colon cancer cells lines HCT 15 and HT 29 (Kim et al., 2008; Lee et al., 2008; Yogosawa et al., 2012). We have previously demonstrated that ginger extract inhibited the growth of HepG2 hepatoma cells and the formation of tumor in liver cancer-induced rats (Mohd Yusof et al., 2008). Ginger was believed to suppress the hepatocarcinogenesis mainly through induction of apoptosis in the cancer cell, as indicated by downregulation of anti-apoptotic Bcl-2 protein and upregulation of pro-apoptotic protein caspase-8 (Mohd Yusof et al., 2008), as well as suppressing inflammation (Jeong et al., 2009; Sang et al., 2009) by inactivating nuclear factor-kappa beta (NF- κ B) (Habib et al., 2008). Other studies have also reported the mechanism of anti-tumour effect of ginger extract via induction of

apoptosis in various human cancer cell lines following treatment with ginger phenolic compounds (Lee and Surh, 1998; Miyoshi et al., 2003).

Two major pharmacological active components found in ginger are 6-gingerol and 6-shogaol. However, shogaols have gained more interest because of recent discoveries revealing their higher anti-cancer potencies over gingerols (Sang et al., 2009). Another component in ginger extract, zerumbone, a sesquiterpene in ginger, has been suggested to induce apoptosis in HCT 116 cell line as indicated by upregulation of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) death receptor (DR) 4 and DR5 (Yodkeeree et al., 2009) while 6-shogaol has been suggested to induce apoptosis in colon adenocarcinoma cell line COLO 205 (Pan et al., 2008). Nevertheless in this study crude ginger extract was used instead of either individual gingerol or shogaol as there is recent evidence suggesting combination of active compounds present in ginger could synergistically enhance the anti-cancer effect of ginger (Brahmbhatt et al., 2013).

Honey is also thought to exhibit its anti-cancer property through several mechanisms. Promoting apoptosis and suppressing cell proliferation are examples of mechanism by which honey could exhibit its anti-cancer property (Jaganathan and Mandal, 2010; Jaganathan et al., 2011; Jaganathan, 2012; Ahmed and Othman, 2013). Despite accumulating evidence suggesting the potential of honeys in combating colorectal cancer, there is not much evidence regarding local Gelam honey having anti-cancer effect and especially colon cancer in particular. There might be differences in the anti-cancer property attributed to differences in the composition of biochemical constituents between different types of honeys (Gheldof et al., 2002; Khalil et al., 2011; Chua et al., 2013). The work documented here is an extension to our previous work in colon adenocarcinoma cell line HT 29 where, we found that Gelam honey exhibited higher chemopreventative effects on HT 29 colon cancer cell line than Nenas honey by inducing apoptosis and suppressing inflammation possibly through its antioxidant property (Wen et al., 2012). These two types of Malaysian honeys have been reported earlier having their antioxidant activities significantly correlated to the content of the phenolic compounds (Hussein et al., 2011).

To our knowledge, this study is the first to demonstrate the anti cancer potential of Gelam honey by inhibiting the proliferation and inducing apoptosis of HCT 116 colon cancer cells. The most possible mechanism of anti-cancer effect of Gelam honey is by arresting cancer cells at G_0/G_1 phase and inducing pro apoptotic proteins as demonstrated in colon cancer HCT 15 and HT 29 cells by Jaganathan and Mandal (2009, 2010). A previous study has suggested that chrysin, a flavanoid component found in honey, is responsible for the anti-cancer property against HCT 116 cells by promoting tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)-induced apoptosis (Li et al., 2011). Another phenolic component of honey, p-Coumaric acid has been shown to induce apoptosis in colon adenocarcinoma cell line HCT 15 (Jaganathan et al., 2013).

Combinations of active compounds from plant extracts

are becoming a new strategy in anti-cancer treatments. Majumdar et al. (2009) have reported the combination of curcumin and resveratrol was effective in inhibiting the growth of colon cancer cells. In the present study, we found that treatment on HCT 116 colon cancer cells with ginger extract and Gelam honey displayed synergism in inhibiting its proliferation as indicated from the CI analysis <1. Treatment with 2mg/ml of ginger reduced the IC₅₀ dose of Gelam honey required for inhibiting the growth of 50% of HCT 116 cells to about one-third (Figures 1B and 1C). However, there was no synergism between ginger extract and Gelam honey in inducing apoptosis of HCT 116 cells (Figure 3).

5-FU, a chemotherapy drug used in the treatment of many cancer types, was shown in earlier studies to interact synergistically with either triptolide or dichloroacetate in killing HT29 cells (Tang et al., 2007; Tong et al., 2011). Combination treatment of 5-FU with dichloroacetate enhanced the 5-FU cytotoxicity and hence reduced the effective drug dose from 798.4µM to 80.0µM (Tang et al., 2007). It was quite interesting to note in our study that the combination of 5-FU with Gelam honey was able to further enhance the capability of 5-FU in suppressing growth of HCT 116 cells. However, we did not notice the same enhancing effect when 5-FU was combined with ginger. On the contrary, ginger extract was able to enhance the apoptosis effect of 5-FU. A study by Ng et al. (2014) showed the enhancement of cytotoxicity of 5FU against colon cancer HT29 cells when treated with *Piper betle* leaf extract.

In summary, our study demonstrates that ginger extract and Gelam honey inhibit proliferation as well as induce apoptosis of a colon adenocarcinoma cell line, HCT 116 *in vitro*. Moreover they displayed synergism in anti-proliferative activity against colon cancer cells when combined. Co-treatment with either ginger extract or Gelam honey also enhanced the anti-tumor effect of 5-FU on the cancer cell line as indicated by increased rate of apoptosis and inhibition of cell growth as compared to treatment with the anti-cancer agent alone.

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