

ORIGINAL RESEARCH ARTICLE



Honey constituents and their apoptotic effect in colon cancer cells

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Summary

Honey finds a vital role in various applications using its antibacterial and anti-inflammatory properties. Our objective was to study the constituents and explore the apoptotic effect of the selected crude honey samples in colon cancer cell lines namely HCT 15 and HT 29. Phenolic content and various functional groups in the honey were analysed using Folin-Ciocalteu method and Fourier Transformed Infrared Spectrophotometer (FTIR). The phenolic content of the honey varied among the different samples. Phenolic content expressed as gallic acid equivalent (GAE) ranged from 29.96 ± 1.54 to 65.08 ± 4.56 mg of GAE/ 100 g of honey. FTIR results indicated the honey sample to be a mixture of numerous compounds including carboxylic acids, aldehydes, alkynes and nitrites. We had also investigated the fluorescence compounds present in the honey after excitation set at 250 nm (emission: 280–750 nm), 290 nm (emission: 305–750 nm). Fluorescence spectroscopy depicted the variation of physio-chemical properties of honey according to their origin as observed by the varying intensity of fluorescent compounds present in the samples. The anti-proliferative effect of the samples in colon cancer cells was explored using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. MTT assay revealed the honey sample containing higher phenolic content showed significant anti-proliferative effect against colon cancer cells. Propidium iodide (PI) staining of 3% honey treated cells indicated a significant number of cells accumulating in Sub-G₁ phase (indicator of apoptosis) after 24 hours. Further, in HT 29 cells, honey elevated the caspase-3 level and displayed typical ladder pattern confirming apoptosis. Most of the drugs used in the cancer are apoptotic inducers, hence apoptotic nature of honey is considered vital.

Keywords: honey, India, apoptosis, colon, cancer, FTIR, fluorescence, spectroscopy

Introduction

Honey finds a role in both domestic and medicinal applications. It has been widely used as sweetener since ancient times. Composition of honey varies depending upon the geographical and the nectar sources of a region. The quality of the honey depends upon its physio-chemical and sensory properties. Hence knowledge about its constituents is essential in judging its quality (Anupama *et al.*, 2003).

Researchers used FTIR to figure out the various compounds and their geographical origin of honey. Further, they also used FTIR attenuated total reflectance spectroscopy (FTIR-ATR) (Sivakesava and Irudayaraj, 2001) and Fourier transform Raman spectroscopy of honey and other sweeteners (canesugar, corn syrup) to identify the adulterants in the honey (Oliveira *et al.*, 2002).

Fluorescent compounds present in the honey give characteristic colour to the honey. Hence analysing honey by fluorescence spectroscopy could hint at the fluorophores present in the honey. Fluorescence spectra had been employed for classifying honey based on their floral source where it had originated. (Ruoff *et al.*, 2006; Karoui *et al.*, 2007)

Characterisation of honey gained importance as it is a common food source for humans. Recent studies revealed that phenolic compounds present in the honey can act as potent anti-oxidants compare to other constituents like Vitamins C and E (Vinson *et al.*, 1995; Cao *et al.*, 1997). Consumption of certain dietary components have been related to several protective effects against cancer and several other disorders such as diabetes and rheumatoid disease (Wollgast *et al.*, 2000). Since honey is one of the common

foods for humans, it prompted us to investigate it as a potential candidate for colon cancer treatment.

Colon cancer is the growing concern for all western countries since the percentage of affected people increases every year according to American Cancer Society. Now this apprehension also holds the Indian sub-continent in its tentacles as there are more than 60,000 new cases of colorectal cancer diagnosed each year. With such a paramount rise in the colon cancer incidence, the use of various natural and synthetic drugs for its prevention has attained remarkable attention in recent years. Most of the drugs used in the cancer treatment are apoptotic inducers. Apoptosis, or programmed cell death, is depicted by distinct morphological features which comprises mainly of cell shrinkage, membrane blebbing, condensation of chromatin, DNA fragmentation which finally leads to the formation of apoptotic bodies (Earnshaw, 1995). Moreover, the expression of various pro-apoptotic and anti-apoptotic proteins were found to be altered during apoptosis. Activation of caspase 3 has been a hallmark during apoptosis (Michael, 2000)

In this investigation, we studied the constituents of the honey using FTIR and fluorescence spectroscopy. Besides, we estimated the phenolic compounds using Folin-Ciocalteu method and assessed the apoptotic effect of the crude honey in colon cancer cells.

Materials and methods

Honey

Four leading brands of honey available in the market were procured and marked as Samples A to D sequentially. All the brands were stored at 4°C and their manufacturing date was under 2 months while performing the experiments. Sample A originated from Kashmir region of India, whereas sample B and sample C were from West-Bengal state. Sample D, was obtained from Uttar Pradesh state of India. According to manufacturers' details, all the honey types were considered multi-floral.

Determination of phenolic content

To determine the phenolic content of honey, the Folin-Ciocalteu method was used. Each honey sample, at a concentration of 0.1 g/ml (in deionized water), was filtered through Whatman No. 1 paper. This solution (0.5 ml) was mixed with 2.5 ml of 0.2N Folin-Ciocalteu reagent (SRL chemicals, Mumbai, India) for 5 min and then 2 ml of 75 g/l sodium carbonate (Na_2CO_3) was added. Before absorbance measurement, the samples were incubated at room temperature for 2 h. The optical density of the samples was measured at 760nm using Spectrophotometer (Perkin Elmer-USA, UV-Vis spectrophotometer, Model: Lambda 45) with methanol as blank. Gallic acid (SRL Chemicals, Mumbai, India) (0–200 mg/l) was used as a standard to produce the calibration curve. The mean of three

readings was used and the total phenolic content was expressed in mg of gallic acid equivalents (GAE)/100 g of honey.

Fourier Transform Infrared analysis

A Thermo Nicolet Nexus 870 spectrometer equipped with a deuterated triglycine sulphate detector was used for analysing honey. Equipment was purged prior to data acquisition to avoid the spectral contribution from atmospheric carbon dioxide and water vapour. Sample absorbance was recorded using computer interface provided and the peaks were analysed separately using Spectrum software version 5.0.1 (Perkin Elmer Inc, Massachusetts, USA)

Fluorescence spectroscopy

Fluorescence Spectrophotometer (Varian Model Cary Eclipse) was used to obtain fluorescence spectral measurements. It consists of monochromators for excitation and emission. A xenon light source provided with a range of fixed width selectable slits, selectable filters, and attenuators were available along with two photomultiplier tubes as detectors. The fluorimeter was connected to an external computer which was provided with the Cary Eclipse Version 1.0 software for data acquisition and graphical display. The fluorescence intensity of all the samples was measured using quartz cuvette.

Cell culture

Human colon cancer cells HT-29 and HCT-15 were maintained as monolayer cultures in DMEM and RPMI-1640 supplemented with 10% fetal calf serum, 1% glutamine and 1% each penicillin and streptomycin. The cells were cultured in a T-25 tissue culture flask for passage and experiments. The plates were maintained at 37°C in 5% CO_2 in humidified air.

MTT assay

Cells in the exponential phase were harvested and counted using a haemocytometer with the aid of trypan blue solution. The cell suspensions were dispensed (150 μl) in triplicate into 96-well culture plates at optimised concentrations of 5000 cells/ well in complete medium. After 48 h incubation of HT-29 and HCT 15 cells in various dilution of honey, cells were incubated with 100 μl of MTT (Sigma-Aldrich, USA) reagent (1 mg/ml) for 5–6 h in the incubator. Briefly, 100 μl of spectrophotometric grade dimethylsulfoxide (DMSO) was added to dissolve the precipitate formed inside the living cells. The coloured formazan product was assayed spectrophotometrically at 570 nm using ELISA plate reader which corresponds to the cell viability.

Cell cycle analysis

Cell cycle analysis of the honey treated colon cancer cells was performed using a FACScan (Becton Dickinson Immunocytometry Systems). Cells were seeded at a density of 100,000 cells per dish and allowed to adhere. After 24 h, cells were synchronized in incomplete

medium supplemented with 2% FBS (for 24 h). Then the cells were treated with 3% (w/v) honey dilutions. Then the cells were washed twice with phosphate buffered saline (PBS) buffer solution by centrifuging at 1200 rpm for 5 min at room temperature. After removing the supernatant, cells were fixed with 70% ethanol for overnight at -20°C. The cell suspension was centrifuged and 1 ml solution of propidium iodide (Sigma-Aldrich, USA) and Rnase A (Sigma-Aldrich, USA) in PBS were added to the pellet and incubated in the darkness for 30 min at 37°C. Finally 5000 cells were analysed using CellQuest Pro software provided with the machine. The DNA content was expressed as sub-G₁, G₀ /G₁, S, and G₂/M phases.

DNA Fragmentation assay

Both control and treated HT 29 cells were lysed in a buffer containing 10 mM Tris (pH 7.4), 150 mM NaCl, 5 mM EDTA, and 0.5% Triton X-100 for 45 min on ice. Lysates were vortexed and then centrifuged at 10,000 g for 20 min. DNA in the supernatant was extracted with an equal volume of neutral phenol:chloroform:isoamyl alcohol mixture (25:24:1) and examined electrophoretically on 2% agarose gels containing 0.1 µg/ml ethidium bromide.

Western Blot

HT 29 cells are grown in 10 cm dish with 10% FBS supplemented DMEM. After 24 h, the medium was decanted and incomplete medium with 2% FBS was added. After 24 h, the cultures were treated with 3% of sample C honey and time response of the honey action was studied from 12, 24 and 48 hours along with the control (without honey treatment). After the treatment period was over, the cells were scraped and transferred to 15 ml falcon tubes and centrifuged at 1000 g for 10 min. The pellet was collected and re-suspended in PBS, transferred in 2 ml microcentrifuge tube and centrifuged again at 10,000 rpm for 5 min. The cell pellet was collected and kept at -80°C. Lysis buffer was prepared freshly and 5 volumes of its were added to the cell pellet and kept until a transparent solution was observed. The micro centrifuge containing the cell lysate was centrifuged at 13,000 rpm for 10 min. at 4°C. The clear supernatant containing protein are transferred to 1.5 ml centrifuge tubes and preserved until further use at -80°C. An equal amount of protein (35 µg) was taken from cell lysate and SDS dye was added before boiling at 95°C for 5 min. The sample (30 µl) was quick spun and loaded in the wells of the SDS-PAGE (10%) gel along with the Rainbow protein marker. It was initially run for 30 min at 60 V and then finally operated at 90 V till the dye front came at the bottom by using BIO-RAD mini protein gel apparatus. Protein was transferred from gel to the nitro cellulose membrane at 1.2 mA/cm² for 1 h and 45 min in transfer buffer. After blocking with 5% BSA, the membranes were incubated with primary antibody (1:1000, dilution, Cell Signalling Technology, Inc. USA) for 1 h at

room temperature and then incubated with a corresponding secondary antibody (1:3000) (anti-rabbit IgG-HRP, anti-mouse IgG-HRP, 1:2000 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) for 30 min. The blots were developed using an ECL detection kit (Sigma Aldrich Inc. USA).

Results

The phenolic content (Table 1) of the honey types varied among the different brands. It was measured by using Folin-Ciocalteu method (Singleton *et al.*, 1999) and expressed as mg of gallic acid equivalent/100 g of honey (GAE). Sample A and C showed almost similar levels of phenolics acid concentration (60-65 GAE) and it was greater than sample D (47 GAE) and sample B (30 GAE).

FTIR analysis indicated absorption bands at almost similar regions for all the samples. It showed peaks at 737, 1027, 1075, 1149, 1360, 1420, 1639, 2126, 2940, 3343, 3371 cm⁻¹ for the sample A. For the remaining samples, peaks were observed with slight variation in their absorption was clearly depicted in the Figure 1. Peaks at 1255, 1256 cm⁻¹ were additionally observed in samples C and D respectively.

Fluorescence spectroscopies of the samples were performed to analyse the fluorophores in the honey. By exciting the samples at 250 nm (Fig. 2a), emission at the range between 280 to 700 nm was investigated. We observed maxima located around 530 to 550 nm depending upon the honey used. Sample C showed higher fluorescence intensity 144.6 arbitrary units (au) compared to other samples. The intensities of the other honey types were found to be 91, 64 and 65 au for samples A, D and B respectively. Figure 2b shows the excitation of samples set at 290 nm which is specific for tryptophan residues present in the proteins. The maxima of the samples were different for each honey. Maximum peaks were located at 512, 568, 554 and 575 nm for samples A to D consecutively. Sample B (19 au) showed the lowest intensity among the four samples. Sample C (45 au) depicted higher intensity which was followed by sample A (30 au) and sample D (29 au).

Table 1. Total phenolics concentration of Indian-honey varieties. Data are the mean of triplicates ± S. E.

Honey samples	Gallic acid Equivalent/ 100g of honey (GAE) (Mean ± S.E)
Sample A	60.46 ± 4.81
Sample B	29.96 ± 1.54
Sample C	65.08 ± 4.56
Sample D	47.10 ± 5.18

Means are significantly different at p < 0.05.

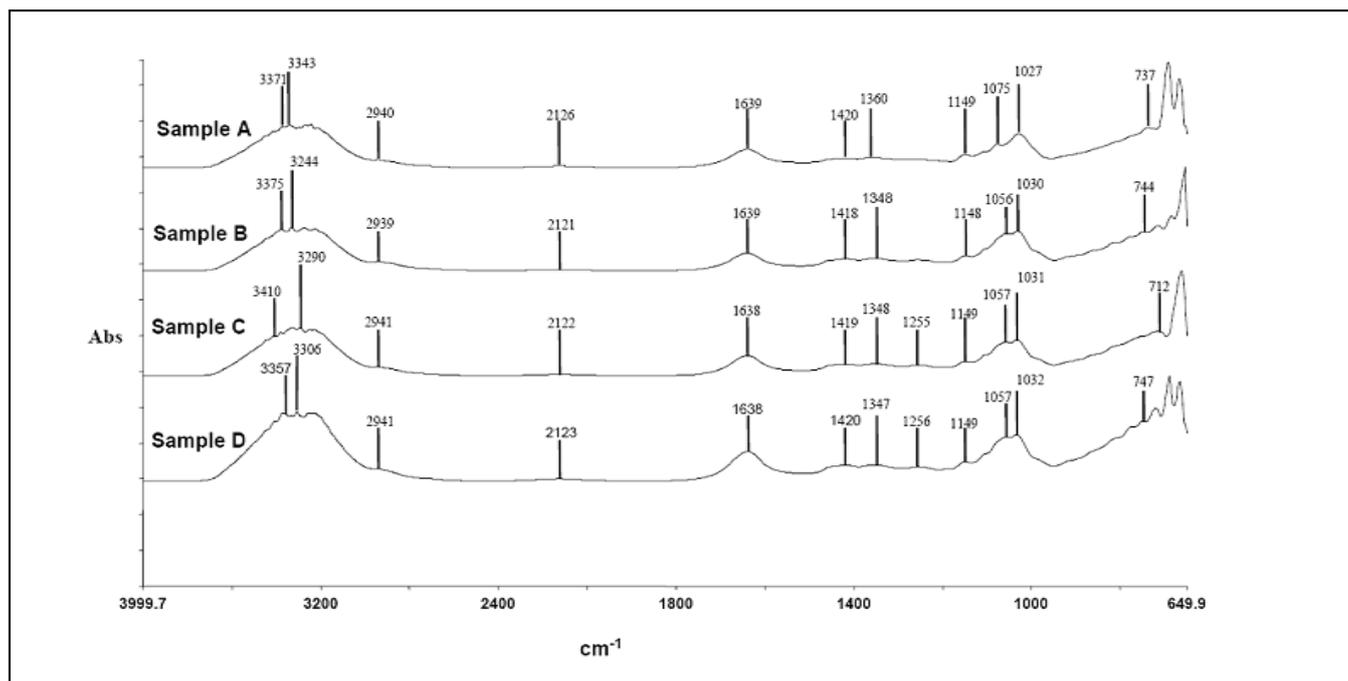


Fig. 1. Fourier transformed infrared spectrophotometer spectrum of the honey samples. For identifying the functional groups, honey samples were spread on the surface of Nexus 870 infrared spectrophotometer and the absorbance were recorded. Absorbance of the samples in the mid-infrared region was plotted using Spectrum software.

The anti-proliferative effect of honey (Fig. 3) was examined using MTT assay. Logarithmically growing colon cancer cells were treated with different concentration of honey diluted in the incomplete medium for 48 h. Cell proliferation was remarkably inhibited depending on the concentration and the type of honey sample used. Sample C and Sample A showed 50% inhibition of both HT 29 and HCT 15 around 2-4% dilutions depending upon cell line. In contrast, Sample D and B showed 50% inhibition of both cell lines approximately between 4 to 8% dilutions. Out of the four samples, Sample B was found to be less potent in inhibiting the cell proliferation.

Cell cycle distribution of honey treated cells was analysed using PI staining. It indicated varying cell population based on their DNA content. Cells treated with 3% showed significant cell arrest at sub-G₁ phase after 24 h. The sub-G₁ arrest was 36, 15, 41, 20 and 18, 6, 30, 16% for samples A to D respectively in HCT 15 (Table 2) and HT 29 cell lines (Table 3). Photomicrographs of cells treated with 3% honey showed typical manifestation of apoptosis like shrinkage, blebbing etc as depicted in Figure 4.

Since Sample C showed significant apoptotic effect, we further examined the apoptosis induction of Sample C in HT 29 cells using Western blot and DNA fragmentation assay. PARP, which catalyses poly-ADPs to the DNA backbone, was cleaved in the treated cells. DNA fragmentation assay of treated cells also displayed typical ladder pattern portraying apoptosis. Moreover caspase-3 level in the treated cells was elevated during the time course study confirming apoptosis (Fig. 5).

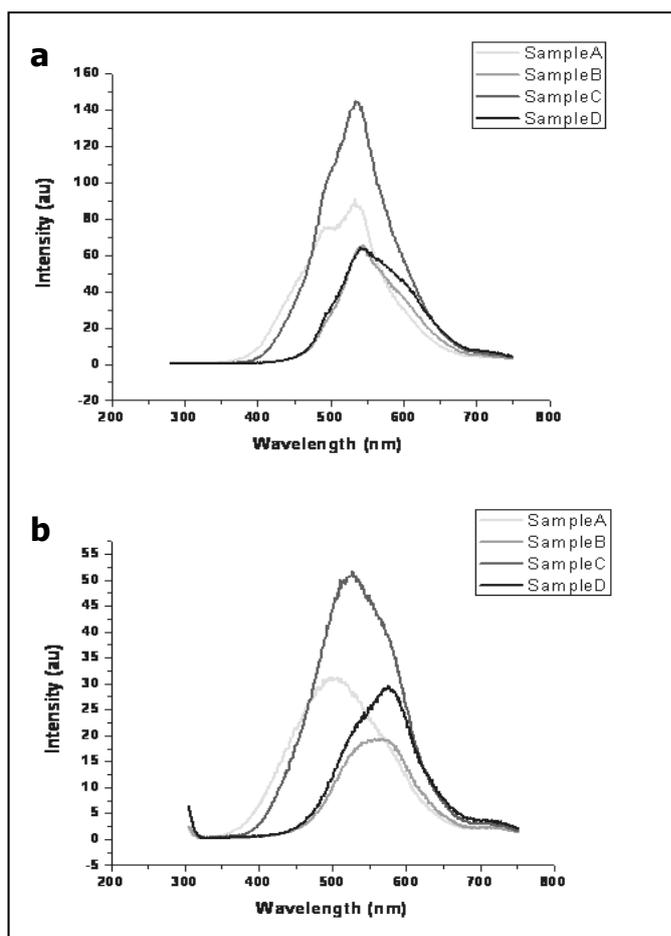


Fig. 2. Fluorescence emission spectra of honey. (a) Normalised spectra obtained after setting excitation at 250 nm (emission: 280–750 nm) (b) Normalised spectra obtained after setting excitation at 290 nm (emission: 305–750 nm)

Table 2. Percentage change of cell cycle as induced by 3% honey treatment in HCT 15 cells after 24 hours

Honey samples	Sub G ₁	G ₀ /G ₁	S	G ₂ /M
Control	2.16 ± 1.87	29.58 ± 3.42	20.52 ± 3.71	36.02 ± 4.21
Sample A	35.94 ± 2.18	12.88 ± 4.92	10.34 ± 5.18	25.54 ± 1.79
Sample B	15.06 ± 0.93	32.32 ± 5.29	3.98 ± 1.21	35.62 ± 3.21
Sample C	41.08 ± 1.58	18.12 ± 2.54	10.02 ± 3.41	21.6 ± 1.29
Sample D	20.08 ± 4.23	17.04 ± 2.12	10.84 ± 5.21	35.48 ± 3.71

Data represents Mean ± S.D.

Table 3. Percentage change of cell cycle as induced by 3% honey treatment in HT 29 cells after 24 hours

Honey samples	Sub G ₁	G ₀ /G ₁	S	G ₂ /M
Control	1.70 ± 1.10	43.56 ± 4.12	26.44 ± 2.65	24.00 ± 3.84
Sample A	18.14 ± 4.78	27.10 ± 1.89	22.88 ± 4.65	28.26 ± 2.91
Sample B	5.74 ± 2.77	41.24 ± 2.25	15.22 ± 3.05	28.02 ± 4.23
Sample C	30.10 ± 4.89	25.22 ± 5.23	16.46 ± 5.12	27.12 ± 4.87
Sample D	16.36 ± 1.47	28.18 ± 3.29	15.28 ± 2.37	35.70 ± 2.67

Data represents Mean ± S.D.

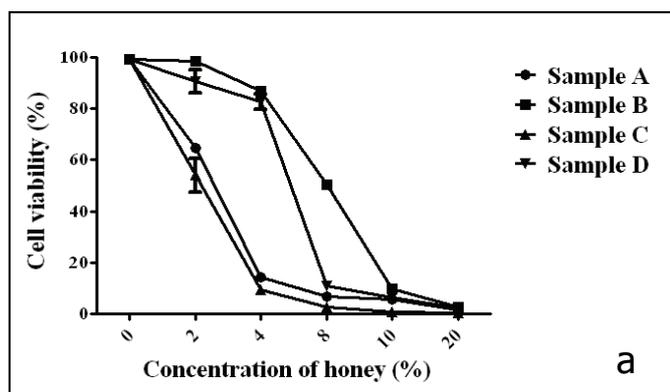


Fig. 3a. Cytotoxic activity of honey on colon cancer cell growth *in vitro* HCT 15 grown in 96-well plates were treated with various concentration of honey diluted in the RPMI media for 48 h. The mean of the percentage cell viability (% of control) along with their standard deviation is indicated.

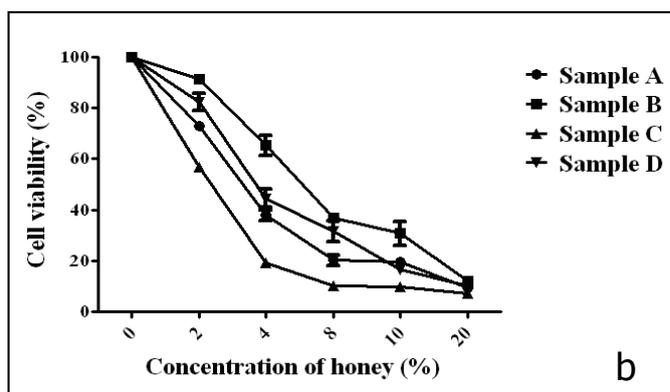


Fig. 3b. Cytotoxic activity of honey on colon cancer cell growth *in vitro* HT 29 grown in 96-well plates were treated with various concentration of honey diluted in the DMEM media for 48 h. The mean of the percentage cell viability (% of control) along with their standard deviation is indicated.

Discussion

The average phenolic acid content of the Indian honey using Folin-Ciocalteu method posed similar values as those claimed by the French, Greek, Slovenian and Burkina Faso honeys (Amiot *et al.*, 1989; Kefalas *et al.*, 2001; Meda *et al.*, 2005; Jasna *et al.* 2007). Although lower phenolic content in sample B may be due to the variation in the floral source, it may also be, to a modest extent, attributed to storage and processing conditions (Gheldof *et al.*, 2002; Turkmen *et al.*, 2005).

FTIR analysis portrayed the presence of various absorption bands starting from 3500 to 700 cm⁻¹. The bands in the absorption zones ranging from 1500–800 cm⁻¹ represented the three major sugars namely glucose, fructose and sucrose in the honey. Wave numbers ranging from 900–750 cm⁻¹ were attributed to the anomeric region which is a characteristic of saccharide configuration. C-O and C-C stretching configurations were assigned for the bands in the region between 1153–905 cm⁻¹. Region between 1474 and 1199 cm⁻¹ were assigned to O-C-H, C-O-H and C-C-H bending modes.

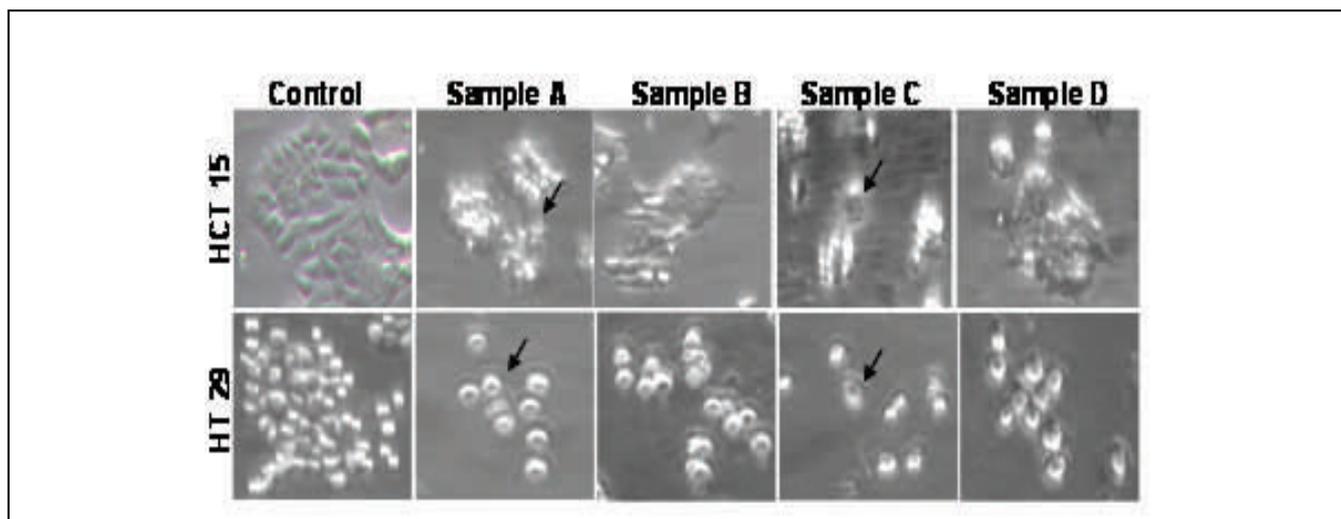


Fig. 4. Photomicrograph of honey treated colon cancer cells. Upper and lower panel shows HCT 15 and HT 29 cells treated with 3% honey for 24 h before taking microscopy images. Images at left extreme of both panels were control and the remaining images were treated with honey samples from A to D sequentially. Arrow mark indicates membrane blebbing.

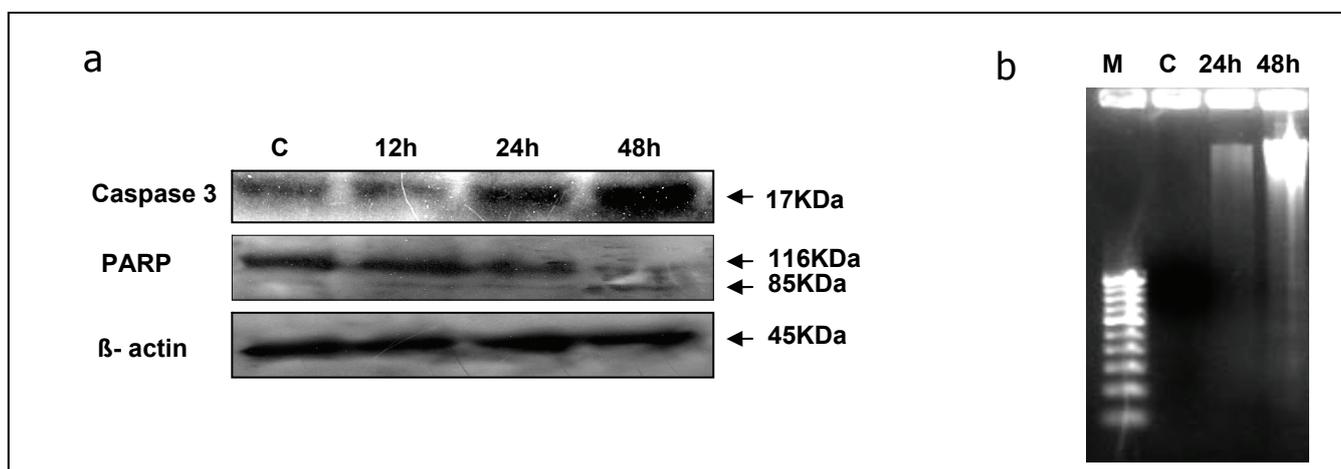


Fig. 5. Western blot and DNA fragmentation of honey treated cells.

(a) Western Blot: HT 29 cells were treated with 3% honey (sample C) for the indicated periods of time followed by subsequent whole cell lysate preparation. Then the proteins were separated by SDS-PAGE and activation of caspase-3, PARP was probed with specific antibodies. (b) DNA fragmentation: Genomic DNA of HT 29 cells were prepared after treatment with 3% honey (sample C) for the mentioned time periods and analysed by 1.5% agarose gel electrophoresis. A photograph of the ethidium bromide stained gel, which is representative of three independent experiments, is shown. M, marker; C, control.

Bands around 1638 cm^{-1} were assigned to the C=C (stretch), C=O (stretch) of esters and carboxylic acids while those bands at 2122, 2123, 2126 cm^{-1} were allotted to the C=C (stretch), C≡N (stretch) of the alkynes and nitrites. Three bands at 2939, 2940 and 2941 cm^{-1} were ascribed to the aldehydes corresponding to their CHO stretching mode. Bands lying between 3200 and 3420 cm^{-1} were due to O-H stretching of carboxylic acids and phenolic constituents. FTIR results indicated the honey samples to be a mixture of compounds namely carboxylic acids, aldehydes, alkynes, esters and nitrites. Although FTIR analysis gave a fair knowledge about the functional groups, it could not differentiate the honey samples based on their origin. Hence, FTIR coupled with principal component analysis (PCA) may be a better tool in identifying the botanical origin (Bertelli *et al.*

2007). This is beyond our objective and the results obtained confirmed the existing literatures on honey composition showing honey as a mixture of carbohydrates, acids, lipids, proteins, minerals and vitamins (White and Kushnir, 1967; National Honey Board, 2007).

Fluorescence spectra obtained at excitation set at 250 and 290 nm hints about the phenolic constituents and tryptophan residues which act as healthy antioxidants. By exciting at 250 nm, the emission range measured from 280 to 750 nm generally gives a picture about the amino acids, nucleic acids, furosine and phenolic constituents. Phenolic constituents in the honey varied according to the geographical origin and many researchers use phenolic compounds as markers for the floral origin of honey.

Emissions at this range were found to be unique for each investigated honey thereby it can be used to differentiate the samples. Sample C showed higher fluorescence intensity which may be accredited to the additional fluorescence contributed by the abundantly found phenolic acids in the sample as previously estimated by Folin-Ciocalteu method. The emission spectra (305 to 750 nm) obtained after excitation set at 290 nm for tryptophan residues were distinct for each sample. Similar to 250 nm excitation, sample C showed higher intensity which further validates that it contains a high amount of fluorophores compared to other samples. The variation of spectra obtained for each sample may be attributed to various factors like environment, pH and solid content of the sample. Hence the excitation spectrum obtained at 290 and 250 nm of the Indian honey varieties may be considered as a fingerprint of that particular honey type as previously reported (Ruoff *et al.*, 2006; Karoui *et al.*, 2007). After assessing the constituents present in the honey varieties we extended our research to find the apoptotic effect of honey in colon cancer cells.

Natural honey has been widely used as antibacterial, wound healing, anti-inflammatory as well as in treating peptic ulcer and gastroenteritis (Molan, 1992). It has been shown that honey can exert an anti-metastatic effect when given before tumour cell injection in animal experiments (Orsolich *et al.*, 2004). The anti-tumour activity of chemotherapeutic drugs such as 5-fluorouracil and cyclophosphamide were facilitated by honey (Gribel and Pashinskiĭ, 1990). We had performed our experiments with crude honey dilution compared to fractionation as we were interested in honey as a whole. There may be a chance of losing some volatile compounds during fractionation, as well as placing a limitation on the study of the synergism between the chemical constituents of honey while fractionating.

Our results indicated that honey could exert significant anti-proliferative effect on the colon cancer cells. The percentage of honey dilution required for growth inhibition varied among the cell lines and the concentration of honey used. This is similar to the observation made by Tarek *et al.* 2003 who had reported anti-proliferative effect of honey in bladder cancer cells. Samples tested showed varying effect on the growth pattern of the cells. Samples C and A repressed the growth of HCT 15 and HT 29 cells more effectively than D and B. The intensities of phenolic acids and tryptophan residues of samples C and A were greater compared to samples D and B. It had been already elucidated that phenolic acids affect the growth of the cancer cells significantly (Chung *et al.*, 2004; Lee *et al.*, 2003). Hence the remarkable anti-proliferative effect of samples C and A may be attributed to the richly available phenolic constituents present in these samples.

DNA content analysis after 24 h honey treatment using flowcytometry showed increasing number of the cell population accumulating in sub-G₁ phase which is an indicator of apoptosis. The sub-G₁ arrest induced by honey was at comparable levels as the arrest induced by caffeic acid phenyl esters (CAPE) in C6 glioma cells (Lee *et al.*, 2003). Our results are in accordance with the recent research published on the anti-neoplastic effect of honey in bladder cancer cells (Tarek *et al.*, 2003). Moreover, the DNA ladder and activation of Caspase-3 were found to be associated with honey induced apoptosis as reported in CAPE induced apoptosis (Lee *et al.*, 2003). Therefore, honey seems to be a plausible candidate for inducing apoptosis in colon cancer cells. However, further experiments concerned with the molecular mechanism of honey-induced apoptosis could promote this candidate in colon cancer prevention.

Conclusion

Four kinds of honey from various geographical locations were analysed. The phenolic content, functional groups and fluorophore present in the honey varied among the samples. Fluorescence spectra obtained after excitation set at 250 nm and 290 nm may be used to classify the honey based on their geographical origin. Further research illustrated that honey-induced apoptosis in colon cancer cells depends upon the concentration of the honey and the cell line tested. Moreover apoptosis inducing ability of all honey types were different. Samples showing higher phenolic content and tryptophan residues displayed significant anti-proliferative potential. Cell cycle analysis suggested honey induces apoptosis by arresting the cells at sub-G₁ phase. Moreover, DNA ladder and activation of caspase-3 were found to be associated with honey induced apoptosis. Since most of the drugs used for cancer therapy are apoptotic inducers, apoptotic nature of honey is considered vital. Detailed investigation of mechanism behind the honey-induced apoptosis is in progress in our laboratory to further validate crude honey as a promising candidate for colon cancer prevention.

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