THE IMPACT OF SUDANESE HONEY ON BREAST CANCER CELLS

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ABSTRACT

Purpose: Breast cancer is the most common cancer in women worldwide. Although breast cancers are generally oestrogen receptor positive initially, a substantial proportion become oestrogen receptor negative. Oestrogen receptor positive breast cancers are associated with a better prognosis than oestrogen receptor negative breast cancers as they are more responsive to hormonal therapy.

The aim of the present study was to assess the inhibitory effect of honey on MCF-7 and MDA-MB-231 breast cancer cell lines.

Methodology: Three different varieties of Sudanese honey collected from different parts of Sudan (south, west and east) were initially tested for their effects on the proliferation of MCF-7 and MDA-MB-231 breast cancer cells. When tested at a 20 times dilution in growth medium, all three varieties of honey produced 40%–85% inhibition in growth of these cells. One particular variety of honey (west) significantly inhibited the

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growth of MCF-7 cells and MDA-MB-231 by 80% ±5 and 50% ±5 respectively. This honey was further characterised. Twenty times diluted honey was treated as follows:

1. Charcoal to adsorb small molecular weight substances;
2. Ether extracted to remove ether-soluble substances;
3. One hour at 65°C to assess temperature-stability of the test substances.

Findings: In conclusion, a large molecular weight soluble component of honey was found to have significant growth inhibitory effects on MCF-7 and MDA-MB-231 breast cancer cell lines. Further analysis is in progress to identify and characterise the substance.

Originality/value: The identification of endogenous inhibitors of breast cancer cells is important. The substance present in honey may possibly have a role to play in the prevention of breast cancer development and progression.

Keywords: oestrogen receptor positive breast cancers; oestrogen receptor negative breast cancers; honey

INTRODUCTION

In 2012, 14.1 million new cases of cancer were reported worldwide. During the same year, the number of people who died from cancer is an estimated 8.2 million (Ferlay et al., 2015). In 2002, 7.6 million people worldwide died of cancer. This is in excess of the 5.6 million deaths from HIV/AIDS, TB and Malaria combined (Stewart and Kleihues, 2003). Breast cancer is the most common cancer in women, both in the developed and less developed countries of the world. It is estimated that 1.7 million new cases were diagnosed in 2012 (Ferlay et al., 2015).

All cancer drugs are associated with side effects, which are often severe and can be unpleasant for patients being treated with these agents. Therefore, there is a great need for continuing research into exploring new affordable alternative of treatments that are less invasive or radical, and to develop treatments that have fewer or milder side effects. Complementary and Alternative Medicine (CAM) is the healing philosophy of medical products and practices, although not integral to standard medicine, such as homeopathy, acupuncture, osteopathy, chiropractic and herbalism[1]. Cancer Research-UK reported that 33% of cancer patients are very interested in using complementary therapies at some time during their illness. This percentage increases among breast cancer patients, reaching up to 50%.

[1]http://www.nhs.uk/Livewell/complementary-alternative-medicine/Pages/complementary-alternative-medicines.aspx

The Agency for Healthcare Research and Quality (AHRQ) reported that in the US during 2011, the total health care cost of cancer was $88.7 billion[2]. On the other hand, a study carried out by the University of Oxford found that the annual cost of all cancers to the UK economy is £15.8bn. Of this, £1.5bn is the annual health care cost of breast cancer patients[3].

Honey is a product produced by honeybees naturally from the nectar collected from flowers of different plants. It is a complex food, mainly composed of carbohydrates (70%–80%), and contains a large number of minor components including organic acids, proteins, free amino acids, vitamins, enzymes, minerals and different other molecules, for example, pigments and flavonoids (White, 1975). A few decades ago, there was a revival of interest in the medicinal properties of honey because it is thought to exhibit a broad spectrum of activities including anti-bacterial, anti-fungal, cytostatic, and anti-inflammatory properties (Jeddar et al., 1985; Hladoń et al., 1980; Yasuko et al., 1984).

The aim of the present study was to study the effects of honey on the growth of MCF-7 and MDA-MB-231 breast cancer cell lines.

MATERIAL AND METHODS

Samples

Three specimens of natural honey (unprocessed) were gathered from different parts of Sudan (East, South and West).

Materials

Dulbecco's Modified Eagle Medium (DMEM), L-glutamine, Foetal Bovine Serum (FBS), Non-Essential Amino Acids (NEAA), sodium bicarbonate ($NaHCO_3$) antibiotic-antimycotic (Penicillin G sodium, 1000 units/mL + streptomycin sulphate, 1000 (g/mL + Amphotericin B, 25µg/mL) and trypsin-EDTA were purchased from Gibco BRL life Technologies Inc.

Phosphate Buffered Saline (PBS) tablets (calcium and magnesium-free) were purchased from ICN Biomedicals Inc. (Costa, Mesa, CA, USA).

Centrifuge filtration molecular weight cut-offs (100KDa, 50KDa, 30KDa, 10KDa and 3KDa, were purchased from Sigma-Aldrich, USA).

Cell Culture

For each experiment, 25-cm^2 tissue culture flasks were seeded with about 5×10^4 cells/flask using medium (3mL) of DMEM, containing 10% FBS, 2mM glutamine, NEAA

[2]http://www.cancer.org/cancer/cancerbasics/economic-impact-of-cancer
[3]http://www.bbc.com/news/health-20222759

and 0.075% $NaHCO_3$ and 1% antibiotic-antimycotic. The cells were allowed to grow to 70% confluency for two days at 37°C in 5% CO_2. The medium was then removed and new DMEM (3mL) containing honey at 20 times dilution in growth medium was added. A similar volume of sterile PBS (pH 7.4) was added to the control flasks. All incubations were performed in triplicate. The cell lines were exposed to the treatments for two days at 37°C in 5% CO_2. At the end of the treatment period, the number of cell nuclei was determined using a Coulter counter.

Stripping of Honey

A modification of the method of Butler et al. (1981) was used to strip the honey of steroids and other small molecules. Charcoal and dextran T70, giving final concentrations of 0.5% w/v and 0.05% w/v respectively, were suspended in 50mL PBS, honey was added to the charcoal dextran for 15 minutes at 4°C, at a spun speed of 3000rpm, 4°C for 10 minutes. The suspension was reconstituted to the corrected volume.

Heated Inactivation of Honey

Honey was heated at 65°C for one hour in a water bath to inactivate heat-unstable Substances in the honey.

Ether Extraction Process

Medium containing honey at 20 times dilution, was extracted with 2 volumes of ether by vortex mixing for 30 second, and the ether phase was discarded.

Reversible Growth

MCF-7 and MDA-MB-231 cells were cultured as above. After honey treatment for 48 or 72 hours, honey DMEM was removed and replaced with fresh DMED. Cells were cultured for a further 72 hours, then cell numbers were counted and compared to PBS controls.

Diluted Honey

A honey treatment was carried out with honey at a range of six concentration (8, 12, 20, 40, 80 and 200 times dilution) in the growth medium.

Centrifuge Filtration Different Molecular Weight Cut-Offs

Honey was filtered using centrifugal filtration according to standard protocols. A range of filter membranes was used to retain molecules having molecular weights of

about 100KDa or more, 50KDa or more, 30KDa or more, 10KDa or more, 3KDa or more, respectively. In all cases, the retentate was used to treat cells, and the filtrate was discarded.

Statistical Analysis

The significance of our findings was tested using prism. Results were considered to be statistically significant when $P < 0.05$.

RESULTS

MCF-7 cell growth was significantly inhibited by three varieties of honey (75% to 50% inhibition: $P < 0.05$). Western honey gives 75%, Eastern honey gives 55% and Southern honey gives 50% as shown in Figure 1.

MDA-MB-231 cell growth was significantly inhibited by three varieties of honey (60% to 40% inhibition: $P < 0.05$). Western honey gives 60%, Eastern honey gives 55% and Southern honey gives 40% (Figure 1).

Western honey showed the highest inhibitory effect in both breast cancer cell lines; therefore this honey was further characterised (see Figures 2 and 3).

The charcoal-dextran stripped honey and heat treated honey had almost the same inhibitory effect as untreated honey. Charcoal-dextran stripped honey inhibited the cell growth of MCF-7 with 80%, while it inhibited the MDA-MB-231 cell growth with 60%. MCF-7 cell growth was significantly inhibited by heated honey up to 75%; on the other hand, MDA-MB-231 cell growth was significantly inhibited with 50% (see Figure 4).

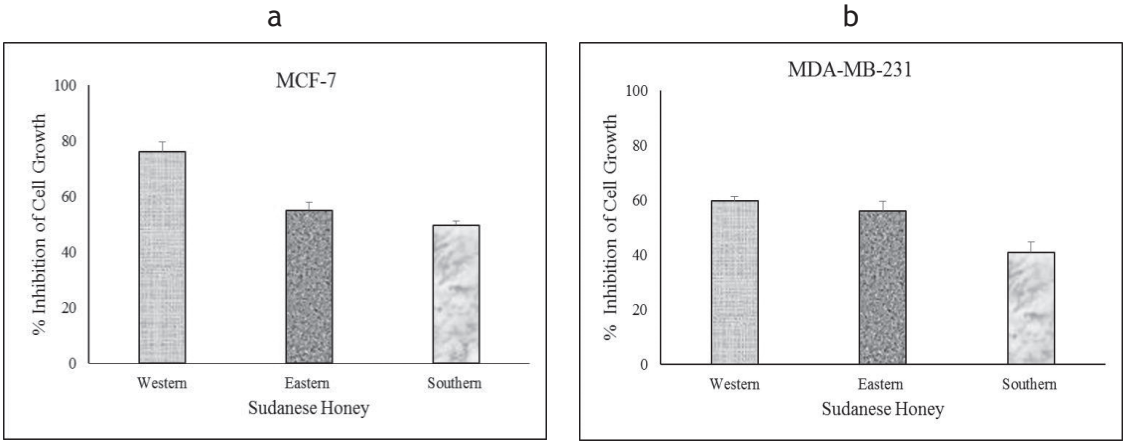

Figure 1 Effects of Honey From Three Sudanese Regions (20 Times final dilution) on Cell Growth of Two Breast Cancer Cell Lines

a) MCF-7

b) MDA-MB-231

a b

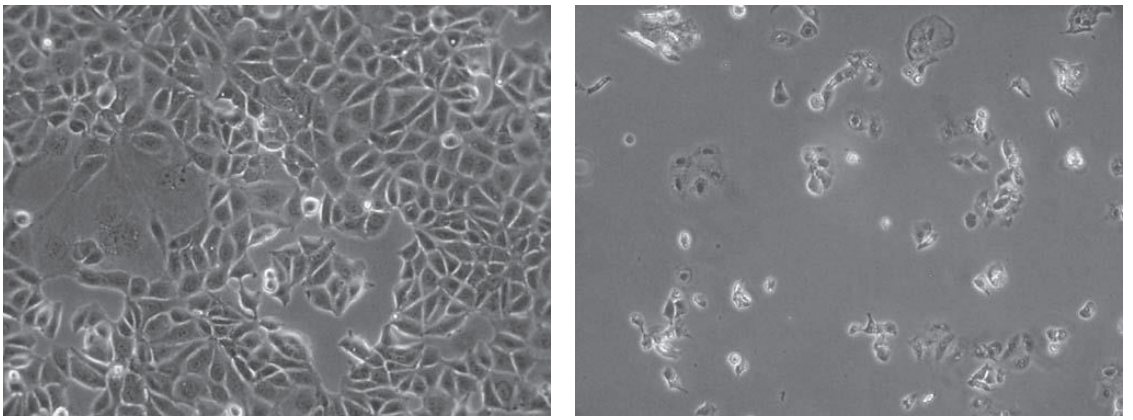

Figure 2 Magnified X10: MCF-7 Breast Cancer Cell Lines Treated for 48 hrs with
a) Phosphatise Buffered Saline (PBS) as a control reference
b) Western Sudanese honey (20 times final dilution)

a b

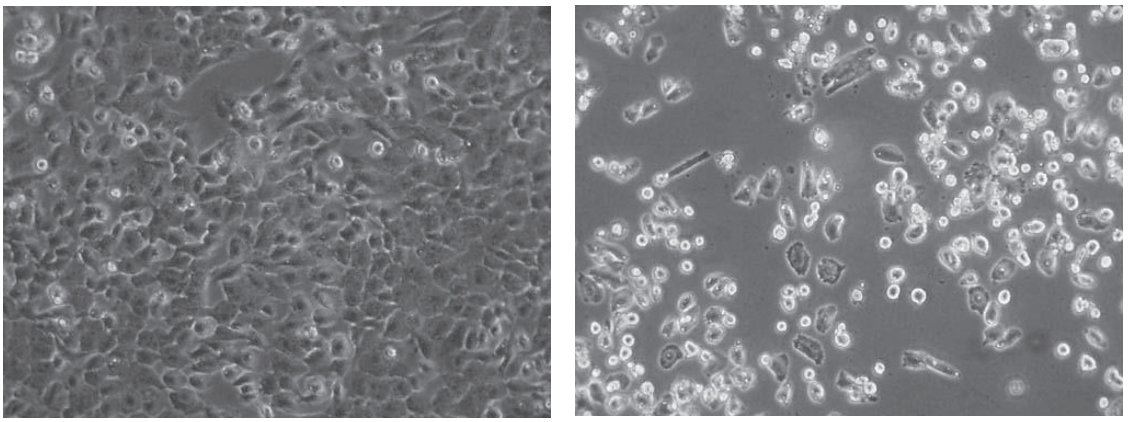

Figure 3 Magnified X10: MDA-MB-231 Breast Cancer Cell Lines Treated for 48 hrs. with
a) Phosphatise Buffered Saline (PBS) as a control reference
b) Western Sudanese honey (20 times final dilution)

Ether extraction honey: the aqueous soluble honey gives the same inhibitory effect of untreated honey. The aqueous soluble honey inhibited the MCF-7 cell growth with 75%, while the ether extraction honey inhibited MCF-7 cell growth with 15%. On the other hand, Aqueous soluble honey inhibited the MDA-MB-231 cell growth with 55%, and ether extraction inhibited the MDA-MB-231 cell growth with 10% (see Figure 5).

Inhibition of cell growth in both MCF-7 and MDA-MB-231 cell lines was found to correlated linearly with increases in honey concentration from 8 to 200 times dilution

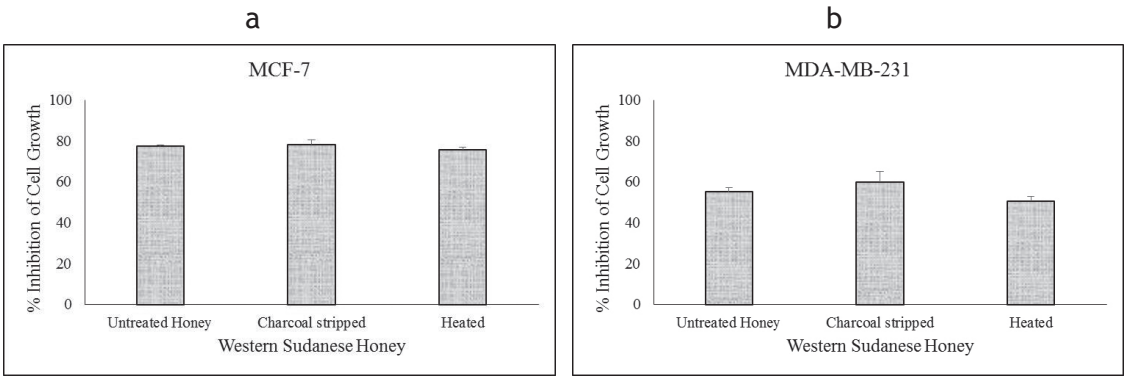

Figure 4 Effects of Charcoal –Dextran Stripped- and Heat-Treated Honey (20 times final dilution) on Cell Growth of Two Breast Cancer Cell Lines

a) MCF-7

b) MDA-MB-231

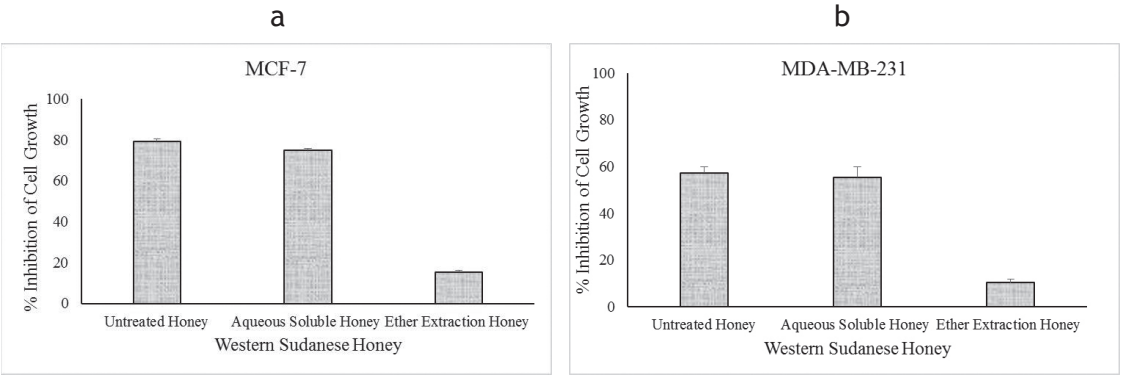

Figure 5 Effect of Ether Extraction Honey (20 times final dilution) on Cell Growth of Two Breast Cancer Cell Lines

a) MCF-7

b) MDA-MB-231

(see Figure 6). MCF-7 cell growth was inhibited at each of the six concentrations of honey tested (8, 12, 20, 40, 80 and 200 times dilution). However, in MDA-MB-231, inhibition of cell growth was observed only with honey concentrations between 8 and 40 times dilution, and stimulated the cell growth at 80 and 200 times dilution.

The effect of honey was tested for the reversible growth and showed that it is irreversible; it increased the percentage of inhibition in both cell lines. MCF-7 cell growth was significantly inhibited with 72% treated for 48hrs, followed by 90% (irreversible inhibition). While inhibited with 80% (72hrs treated), followed by 96% (irreversible inhibition) (see Figure 7). MDA-MB-231 cell growth was significantly inhibited with 51%,

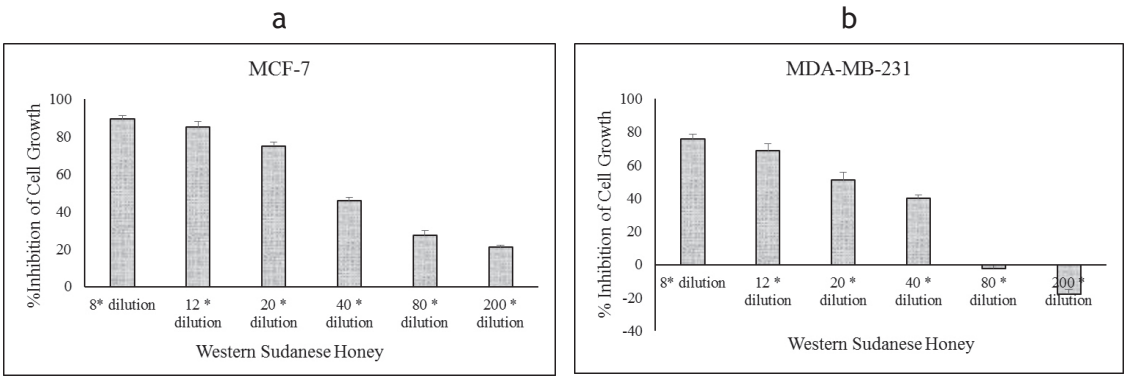

Figure 6 Effect of Six Different Honey Dilutions on Cell Growth of Two Breast Cancer Cell Lines

a) MCF-7

b) MDA-MB-231

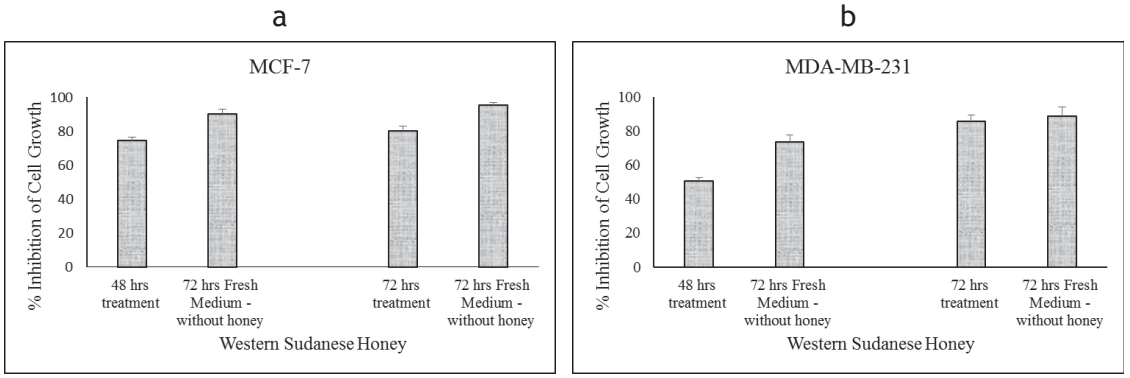

Figure 7 Effect of Honey (20 times final dilution) on Reversible Cell Growth of Two Breast Cancer Cell Lines

a) MCF-7

b) MDA-MB-231

followed by 74% (irreversible inhibition), while inhibited with 86% (72hrs treated) 89% (irreversible inhibition) (see Figure 7).

The components of honey responsible for cell growth inhibition were retained by 100KDa filter membranes and below, but not to filter membranes above 100KDa.

DISCUSSION

Studies by Grible and Pashinskii in 1990, indicated that honey possessed moderate anti-tumour and pronounced anti-metastatic effects in five different strains of rat

and mouse tumours. Furthermore, honey potentiated the anti-tumour activity of chemotherapeutic drugs such as 5-fluorouracil and cyclophosphamide (Gribel and Pashinskiĭ,1990).

In this study the effects of honey on the cell growth of MCF-7 and MDA-MB-231 breast cancer cell lines were examined. Growth inhibition by honey confers a protective effect against the development of breast cancer. A heat stable, water soluble large molecular component of honey was found to have significant growth inhibitory effects on MCF-7 and MDA-MB-231 breast cancer cell lines. The substance present in natural honey may possibly have a role to play in the prevention of breast cancer development and progression.

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BIOGRAPHY

Rasha Alhaj is an inventor; she discovered the substance in honey that inhibits the growth of breast cancer cells. She is the founder of "The Amal Initiative", a project to bring healing and hope to cancer patients. In 2015, Rasha received the Afrabia Afro-Arab Youth Award. She has a BSc in Biological Science from UAE University, an MSc in Chemical Pathology from the University of Putra, Malaysia, and an MRes in Bioengineering from Imperial College, London.

Dr. Alan Purohit is a Visiting Consultant reader Investigative Medicine, Imperial College London. He has Qualifications in BSc, MSc, PhD FRCPath. He is an enthusiastic and dedicated scientist in the fields of cancer aetiology and therapeutics with over twenty years' academic – industrial experience in Oncology Drug Discovery & Women's Health. He is the Pioneer and International lead in the development of Steroid Sulfatase Inhibitors. His research interests have focussed mainly on the regulation and control of oestrogenic steroid synthesis in normal and malignant breast tissues. Research into the regulation of the enzymes involved in oestrogen synthesis in relation to the growth and development of breast tumours, has led to more than 180 published high-impact papers and more than 27 patents."