



Journal of Medical Sciences

ISSN 1682-4474

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Research Paper

J. Med. Sci., 2 (2): 55-58
March-April, 2002

JMS (ISSN 1682-4474) is an international, peer-reviewed scientific journal that publish original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued six times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Asmah Rahmat
Department of Nutrition and Health Sciences,
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia,
43400, Serdang, Selangor,
Malaysia

E.mail:asmah@medic.upm.edu.my

Antiproliferative Activity of Pure Lycopene Compared to Both Extracted Lycopene and Juices from Watermelon (*Citrullus vulgaris*) and Papaya (*Carica papaya*) on Human Breast and Liver Cancer Cell Lines

Asmah Rahmat, ¹Rozita Rosli, Wan Nor I'zzah Wan Mohd. Zain, Susi Endrini and Huzaimah Abdullah Sani

Lycopene is one of the major carotenoids in the diet and is believed to have a number of health benefits including anticancer properties. This investigation was conducted with the aim of screening the potential anticancer properties of pure lycopene and of both extracted lycopene and juices from watermelon and papaya. Two different types of human tumor cell lines, HepG2 (liver cancer cell line) and MDA-MB-231 (breast cancer cell line) were used for the evaluation of cytotoxicity effects. Chang liver cell line that is the transformed cell for the liver was used for comparison. The cells treated with pure lycopene, extracted lycopene and juices from both watermelon and papaya were maintained and incubated at 37°C in 5% CO₂ for five days. The Microculture Tetrazolium salt (MTT) assay was carried out in this investigation, to determine the cell viability. Pure lycopene was found to cause 50% cell death (IC₅₀) of HepG2 cells at a concentration of 22.8 µg ml⁻¹, while papaya juice at a concentration of 20 mg ml⁻¹. In the case of MDA-MB-231 cells, the IC₅₀ of the watermelon was 11.3 mg ml⁻¹. The other samples including extracted lycopene samples did not show any effect in the cell viability. The findings of this study showed that pure lycopene and papaya juice may have anticancer properties upon liver cancer cell line (HepG2), while watermelon juice had anticancer properties against breast cancer cell line (MDA-MB-231). The juices seemed to be more effective than the extracted lycopene samples in inhibiting cancer cell growth.

Key words: Pure lycopene, extracted lycopene, juices from watermelon and papaya, HepG2, MDA-MB-231, MTT assay, cell viability

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Department of Nutrition and Health Sciences and ¹Department of Human Growth and Development, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia

Introduction

Cancer has become one of the leading causes of death in the world due to the increasing number of cancer patients. Of the estimated 9 million new cancer cases every year, more than half are in developing countries. Majority of the patients are incurable by the time their disease is diagnosed (Sporn, 1996). Many drugs have been synthesized chemically in order to heal this disease, although some of these drugs are proven to be effective in treating cancer but their side effects could not be prevented. This is because most of the anticancer agents suppress the cells and normal tissues, which are actively proliferating such as bone marrow, reproductive tissue and hair follicles. A diet rich in carotenoid-containing foods is associated with a number of health benefits.

Nowadays, interest in lycopene is growing rapidly following the recent publication of epidemiological studies implicating lycopene in the prevention of cancers and other diseases (Steven and Clinton, 1998). Lycopene is an antioxidant that once absorbed by the body, helps to prevent and repair damaged cells. Antioxidants are compounds that fight free radicals in the body and have been shown to inhibit DNA oxidation that can lead to some cancers (DiMascio *et al.*, 1989; Halliwell, 1994). The human body does not produce lycopene, but it is readily available through the diet. Minor sources include guava, rosehip, watermelon and pink grapefruit. About 85% of dietary lycopene comes from tomatoes and tomato products such as juice, soup, sauce, paste and ketchup (Mangels *et al.*, 1993). Research confirms that lycopene from tomatoes is absorbed much better in the bloodstream if it is first processed. As lycopene levels in the blood increase, the levels of oxidized compounds decrease (Gerster, 1997).

A number of studies have indicated that a lycopene-rich diet lowers the risk of certain chronic diseases such as cancer and heart diseases (Block *et al.*, 1992; Ziegler, 1989), in that dietary lycopene acts as an antioxidant, as lycopene levels in the blood go up, the levels of oxidized lipoprotein, protein and DNA compounds go down. This, in turn, helps to lower the risk of cancer and heart disease. Watermelon (*Citrullus vulgaris*) is a tendril-climbing, annual, herbaceous plant that is related to the muskmelon, pumpkin, squash and gourd families.

Watermelon is also one of the leading sources of lycopene, second only to the tomato. It has high lycopene content especially in the red flesh which has about 2.30-7.20mg lycopene 100 g⁻¹ (Nguyen and Schwartz, 1999). Papaya (*Carica papaya*) is considered a part of the Caricaceae family. Papaya a low-caloried fruit also has a high lycopene content, especially in the red flesh which has about 4.10 mg lycopene 100 g⁻¹ (Nguyen and Schwartz, 1999). Researches have been conducted to studying the relationship between dietary lycopene, oxidative stress and cancer risk. Their studies will further examine the role of lycopene as an antioxidant in preventing cancers of the breast (Potischman *et al.*, 1990; Garland *et al.*, 1993; Nagasawa *et al.*, 1995; Levy *et al.*, 1995), prostate (Giovannucci *et al.*, 1995; Mills *et al.*, 1989), colon (Narasawa *et al.*, 1996), cervix (VanEewyck *et al.*, 1991), lung and digestive tract (Franceschi *et al.*, 1994; Bjelke, 1974; Correa *et al.*, 1985; Buiattii *et al.*, 1989) as well as cardiovascular disease and degenerative diseases of the eye. Preliminary studies have suggested that carotenoids are unstable when provided to cells in typical oxygen-rich, warm environments over several days. However lycopene was found to protect cultured hepatocytes against carbon tetrachloride (CCl₄) injury and death (Kim, 1995). Several investigations have reported the antiproliferative effects of lycopene against cancer cells in culture such as breast cancer (Stahl and Sies, 1996).

Lycopene has a unique structural and chemical features (Fig.1) that may contribute to specific biological properties. Data concerning lycopene bioavailability, tissue redistribution, metabolism, excretion and biological actions in experimental animals and humans are beginning to accumulate although much additional research is necessary.

In this experimental work, besides pure lycopene, extracted lycopene and juices from watermelon and papaya were also being used to determine whether those fruits also contain healing or preventive properties against breast and liver cancers.

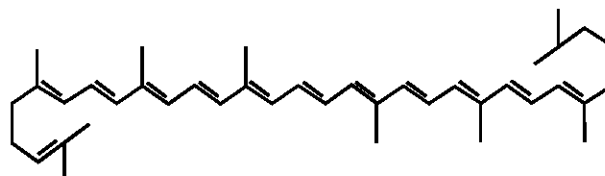


Fig. 1: Structure of lycopene

Lycopene
Molecular Weight : 536.89
Molecular Formula : C₄₀H₅₆

Materials and Methods

Effect of lycopene on proliferation of HepG2 and MDA-MB-231 cells: The cell lines comprising of MDA-MB-231 (breast cancer cell line), HepG2 (liver cancer cell line) and Chang liver cell line were obtained from American Type Culture Collection, USA. The growth medium for the MDA-MB-231 cell line was Dulbecco's Modified Eagle's Medium (DMEM) (GIBCO BRL, Life Technologies, USA), while the growth medium for HepG2 and Chang liver were Minimum Essential Medium (MEM).

Fetal Bovine Serum, penicillin-streptomycin and trypsin were from GIBCO BRL, Life Technologies, USA, while trypan blue was acquired from Sigma Chemical Co., St. Louis, USA. The MTT kit was obtained from Boehringer Mannheim GmbH, Germany.

Lycopene samples : Pure lycopene (90-95%) (from tomatoes was obtained from Sigma Chemical Co. St. Louis, USA) was dissolved in 1 ml of benzene (C₆H₆). Fruit slices (watermelon and papaya) were weighed, squeezed and strained to obtain juice. 1 ml of the juice was centrifuged at 16,800 rpm for 15 minutes by using an ultracentrifuge (Beckman, USA). The Juice was then transferred into an eppendorf tube and lycopene was extracted. The extraction was performed according to the Manual of Analysis of Fruit and Vegetable Products (Ranganna, 1997).

Cell Culture: The cancer cell lines were cultured in their respective media with 5% Fetal Bovine Serum (FBS), 100 IUml⁻¹ penicillin and 100µg ml⁻¹ streptomycin by using 25 cm² flasks in a 37°C incubator with 5% CO₂.

Subculturing: The culture medium was replaced with new medium. The flask was then rinsed with PBS-EDTA to wash the cells. Following that, PBS-EDTA was removed, then 0.75 ml trypsin was added and the flask was incubated at 37°C and 5% CO₂ for 3 to 5 minutes. The flask was then 'knocked' to let the cells detached from the lower part of the flask, trypsin was removed. Finally, 10-14 ml medium was added and divided into two parts, half of the culture was then transferred into a new flask. Cells were grown until they were confluent, then harvested and the amount of viability was counted using the trypan blue method and a hemocytometer. 100 µl medium that containing 1 × 10⁵ cell ml⁻¹ suspension cells were placed into each well of a 96 well plate. The plate was then incubated overnight at 37°C with 5% CO₂. The following day, the medium was replaced (Freshney, 2000).

Cell proliferation assay: Cells were grown in a 96 well microliter plate (Nunc, Denmark) in a final volume of 100µl culture medium per well. The cells were then treated with pure lycopene, extracted lycopene and juices from watermelon and papaya were maintained at 37°C with 5% CO₂ for 24 to 96 hours. After the incubation period, 10 µl of the MTT labeling reagent (final concentration 0.5 mg ml⁻¹) (Roche Diagnostics, USA) was added to each well. The microliter plate was then incubated for a further 4 hours at 37°C with 5% CO₂. Then, 100 µl of the solubilization solution was added into each well. The plate was allowed to stand overnight in the incubator at 37°C with 5% CO₂, following which cell viability was measured using an ELISA reader (EL_x 800) at a wavelength of 550nm.

Results

The Microculture Tetrazolium Salt (MTT) assay was used to measure the amount of cell viability. The antiproliferative activity of pure lycopene and in both extracted lycopene and juices from watermelon and papaya on human tumor cell lines, HepG2 and MDA-MB-231 was investigated. Chang liver cell line, which is the normal cell culture for the liver, was used for comparison. The percentage of cell viability was measured by comparing the optical density (OD) against the control. The antiproliferative activities are presented as percentage of cell viability versus concentration. Pure lycopene was found to cause 50% of HepG2 cell death at a concentration of $22.8 \mu\text{g ml}^{-1}$ (Fig. 2), while papaya juice was

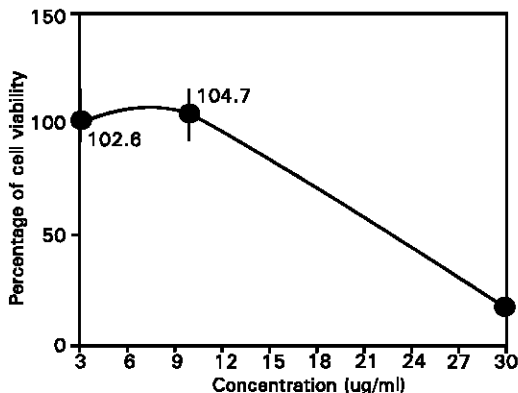


Fig. 2: Effect of pure lycopene on the viability of HepG2 cell line $\text{IC}_{50} = 22.8 \mu\text{g ml}^{-1}$

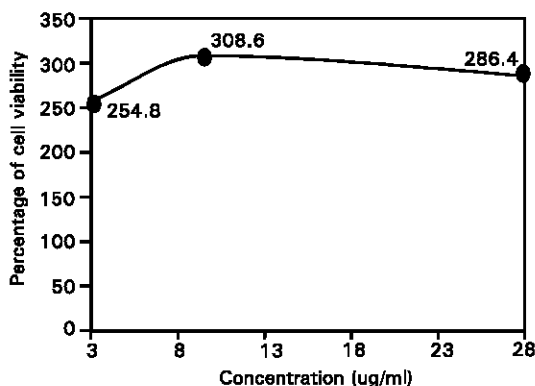


Fig. 3: Effect of pure lycopene on the viability of Chang Liver cell line

detected to cause 50 % cell death of HepG2 at 20 mg ml^{-1} (Fig. 4). Watermelon juice was found to cause 50 % cell death of MDA-MB-231 at a concentration of 11.3 mg ml^{-1} (Fig. 6). However, the extracted lycopene samples did not show any difference in the cell viability. In fact, growth promoting effects were seen. The effect of pure lycopene on the proliferation of Chang liver cell was also detected and the results show that lycopene did not cause 50% of the cell death of Chang liver (normal cell) line (Fig. 3).

Fig. 4 illustrates that papaya juice was detected to cause 50 % of the cell death of HepG2 cells at a concentration of 0.715 v^{-1} or equals to 20 mg ml^{-1} whereas this juice did not cause 50% of the cell death of Chang liver (normal cell) line (Fig. 5). Watermelon juice was found to cause 50% cell death of MDA-MB-231 at a concentration of 0.917 v^{-1} or equals to 11.3 mg ml^{-1} (Fig. 6).

Discussion

Lycopene, which is one of the carotenoids, is endowed with powerful anticancer properties and is now considered to be

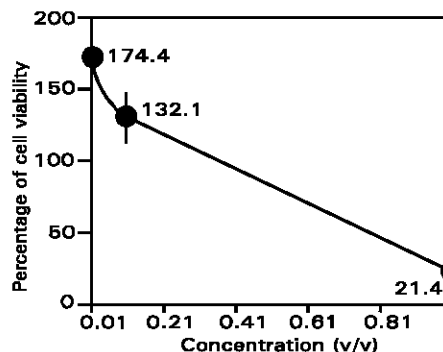


Fig. 4: Effect of papaya juice on the viability of Hep G2 cell line $\text{IC}_{50} = 20 \text{ mg ml}^{-1}$

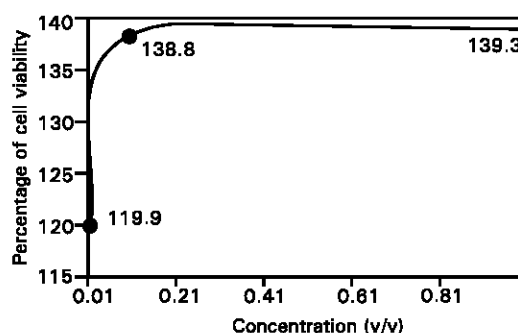


Fig. 5: Effect of papaya juice on the viability of Chang Liver cell line.

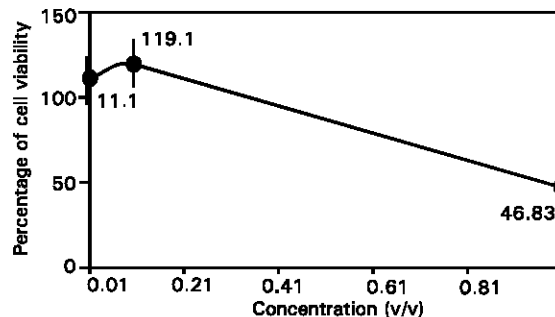


Fig. 6: Effect of watermelon juice on the viability of MDA-MB-231 cell line $\text{IC}_{50} = 11.3 \text{ mg ml}^{-1}$

potentially important for the prevention of cancers and other diseases. Lycopene can be found in a diverse array of fruits and vegetables such as tomato, watermelon, papaya and others especially in the red flesh. Watermelon and papaya are examples of the rich source of lycopene. The findings from this experimentation showed that pure lycopene exhibited strong antiproliferative activity when compared to both, extracted lycopene and juices from watermelon and papaya. However, pure lycopene inhibited only the cell proliferation of HepG2, lycopene did not show antiproliferative activity towards MDA-MB-231 at the same concentration ($100 \mu\text{g ml}^{-1}$) in which it inhibited the cell proliferation of HepG2. Although pure lycopene did not show antiproliferative activity towards MDA-MB-231, watermelon juice, which contains high lycopene, displayed effect in the proliferation of MDA-MB-231. This can be used to support Levy *et al.* (1995), who noted that lycopene inhibited proliferation of human MCF7 mammary cancer cells. But, in this study, a different type of breast cancer cell line was used. MCF7 is an estrogen receptor positive, while MDA-MB-231 is an estrogen receptor negative cell

line. MDA-MB-231 was used to avoid the hormonal effect. Therefore, it was shown that lycopene could also inhibit the proliferation of MDA-MB-231 cancer cells. However, watermelon juice did not show any effect on the cell proliferation of HepG2. Several studies have shown that insignificant or no association between either lycopene from the diet (Freudenheim, 1996), and concentrated lycopene (London *et al.*, 1992) and breast cancer risk. However, others have found a relationship between lycopene that deposited in breast tissue and breast cancer risk (Zhang *et al.*, 1997). A recent study of samples (Dorgan *et al.*, 1998) were analyzed to evaluate the relationship of levels of carotenoids (including lycopene), selenium and retinol with breast cancer. Only lycopene was found to be reducing the risk for developing the breast cancer. Other carotenoids were not found to be associated with reduced breast cancer risk. However long-term studies are needed to establish the protective role of lycopene in breast cancer. In addition, the sample which exhibited antiproliferative activity on HepG2 cell was papaya juice. Papaya juice was found to cause 50% of the cell death at the concentration of 20mg ml⁻¹ but did not show any difference effect on the cell viability of MDA-MB-231. Extracted lycopene from watermelon and papaya juices did not show any difference in the cell viability. In fact, growth promoting effects were seen. Chang liver cell line which is the normal cell culture for liver was used as comparison and the IC₅₀ could not be obtained. This showed that lycopene is a selective anticancer agent where, it only caused cell death in HepG2 (liver cancer cell line) and not in the Chang liver. The unexpected results especially for the extracted lycopene samples obtained may be due to multiple factors such as the microbial contamination during the treatment or because lycopene could not be stored too long since it can easily be oxidized and is very sensitive to light and render it inactive. The fact that the extracted lycopene samples did not show any effect in cell proliferation may also be due to the active lycopene that was not successfully extracted during the extraction process. This research work showed that pure lycopene also had antiproliferative activity towards liver cancer cell line. This is a significant finding because there has been no previous published data which showed that lycopene could also prevent or inhibit proliferation of HepG2 (liver cancer cell line). The findings of this study showed that pure lycopene and papaya juice have anticancer properties against liver cancer cell line (HepG2), while watermelon juice has anticancer properties against breast cancer cell line (MDA-MB-231). It was observed that higher the concentration of lycopene, higher the amount of cell death. The juices seemed to have stronger antiproliferative activity than the extracted lycopene samples in inhibiting cancer cell growth. The extracted lycopene from watermelon and papaya juices displayed no difference in the cell viability whereas growth promoting effects were seen instead.

Acknowledgment :This project was funded by a UPM short-term grant (1999)

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MS received 19th November, 2001; accepted 25th January, 2002