Chemistry Research Journal, 2017, 2(6):262-266

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Evaluation of Extract of Lycopene-Rich Tomatoes on the Peroxidation of Rat Liver Microsomes

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Abstract Lycopene is the important pigment responsible for the characteristic red color of tomato, it has attracted attention due to its biological and physicochemical properties, especially related to its effects as a natural antioxidant. The objective of this study was to investigate the antioxidant effect of the lycopenes obtained from the extract of tomato on the peroxidation of hepatic microsomes membranes. Rat liver microsomes where incubated with different concentrations of extract (2, 4, 6, 8 µg) in an in vitro non-enzymatic ascorbic acid-Fe+2 system in order to determine the oxidative effect on membranes and to quantify peroxidation level in standardized conditions. Peroxidation was quantified in a liquid scintillation counter Packard 1900 TR by chemiluminescence in cpm (counts per minute). Microsomal membranes without extract were used as controls. Analyzing the effect of lycopene (tomato extract), was observed that the total cpm/mg protein originated from light emission: chemiluminescence, was statistically lower in samples obtained from lycopene group than in the control group (without lycopene), the antioxidant effect found was not dependent concentration.

Keywords Lycopene, Antioxidants, Peroxidation, Chemiluminescence, Microsomes

Introduction

Lycopenes are hydrophobic compounds that are within the group of carotenoids, are present in some fruits, vegetables and fungi therefore are only obtained from natural sources, the animals do not synthesize lycopene in your body, which is why importance its addition in the diet [1]. One of the most important sources of lycopene is tomato, the amount of lycopene present in it will vary depending on maturity, genotype, processing as well as there will be differences in the assimilation and the use of it [2].

There is a direct relationship between tomato consumption and decreased risk of suffering from different diseases [3]. This is attributed to the antioxidant power possessed by lycopene, reducing the amount and production of free radicals. Lycopene is the antioxidant with the highest potency to inhibit singlet oxygen in vitro, compared to other carotenoids [4]. Due to its conjugated double bond systems, lycopene is a system rich in electrons, susceptible to being attacked by electrophilic reagents, because of this the lycopene can bind singlet oxygen and free radicals as

well as hydroxyl radicals and various peroxide radicals. This behavior is the basis of the antioxidant action in biological systems, so it is considered a chemopreventive agent [2].

According to previous studies it has been shown that lycopenes, after oral administration, undergo a systemic distribution in particular to organs and products that have a high number of low density lipoprotein receptors and high uptake of them such as the liver, adrenal glands, testicles, as well as the spleen; and has been found in the prostate too [1]. And recently it has been in great demand as a food additive and a natural antioxidant. Additionally, lycopene also exhibited potentneuroprotective, anti-inflamatory, anti-proliferative, maintenance of normal cell metabolism, cognition enhancing properties, regulating blood lipid metabolism [5].

It is demonstrated in an analysis, that healthy individuals supplemented orally with tomato products for 15 days presents a significant increase in the delay of the oxidation of lipoproteins [1]. Found that the consumption of tomato pure for 14 days increased the resistance of lymphocytes against oxidative DNA damage [6].

These studies warn about the importance of tomato consumption to be able to generate protection against oxidative damage in vivo, thus generating the prevention of mutations in the association to the initiation and progression of cancer [7].

Material and Methods

Experimental

Female Wistar AH/HOK rats, 7 weeks old, weighing 120-137 g were used. All rats were fed commercial rat chow and water ad libitum Female Wistar AH/HOK rats were obtained from Laboratory Animal Facility, Facultad de CienciasVeterinarias, Universidad Nacional de La Plata. BSA (fraction V) was obtained from Wako Pure Chemical Industries Ltd, Japan. L (+) ascorbic acid, dimethyl sulfoxide and methanol were from Merck Laboratories. All other reagents and chemicals were of analytical grade from Sigma-Aldrich.

Solanum lycopersicum (SL) extract preparation

Two grams of tomato dried were milled until plant material passed through a 2 mm screen and put in a flask together with 50 ml methanol for extraction under mechanical agitation at 870 rpm and dim light during 12 h. After extraction SL extract was submitted to vacuum filtration and then concentrated using a rotary evaporator (Senco Ltd, Zhong Shan Nan Yi Rd., Shanghai, China.) until total evaporation of the solvent. A residue of 0.8 g was obtained, which was re-suspended in 20 ml DMSO.

The rats were euthanized by cervical dislocation and the liver was rapidly removed, cut into small pieces and extensively washed with 0.15 M NaCl. A 30 % (w/v) homogenate was prepared in a 0.25 M sucrose solution, 10 mMTris-HCl pH 7.4 using a Potter-Elvejhem homogenizer. The homogenate was spun at 10,000 x g for 10 min. The supernatant (30 ml) was applied to a Sepharose 4B column (1.6 x 12 cm) equilibrated and eluted with 10 mMTris-HCl pH 7.4, 0.01 % NaN_3. The microsomal fraction appearing in the void volume (10-12 ml) was brought to 0.25 M sucrose by adding solid sucrose. All operations were performed at 4 °C and under dim light. The quality of microsomal preparation is similar in composition as regards concentrations and activities of certain microsomal enzymes to that obtained by Ultracentrifugation [8].

Microsomes Peroxidation

Rat liver microsomes were incubated with different concentrations of extract (2, 4, 6, 8 µg) in an in vitro non-enzymatic ascorbic acid-Fe^{+2} system in order to determine the oxidative effect on membranes and to quantify peroxidation level in standardized conditions. Peroxidation was quantified in a liquid scintillation counter Packard 1900 TR by chemiluminescence in cpm (counts per minute). Microsomal membranes without extract were used as controls.

Chemiluminescence and peroxidation were initiated by adding ascorbate to microsomes [8]. The microsomes (0.5 mg microsomes protein) with addition of SL total extract (2, 4, 6 and 8 µg) were incubated at 37 °C with 0.01 M phosphate buffer pH 7.4, 0.4 mMascorbate, final vol. 1 ml. Phosphate buffer is contaminated with enough iron to provide the necessary ferrous or ferric iron (final concentration in the incubation mixture was 2.15 µM) for peroxidation [9]. Microsome preparations, which lacked ascorbate, were carried out simultaneously. Membrane light

emission was determined over a 180 min period, chemiluminescence was recorded as count per minute (cpm) every 10 min and the sum of the total chemiluminescence was used to calculate cpm/mg protein. Chemiluminescence was measured as counts per min in liquid scintillation analyzer Packard 1900 TR equipment with a program for chemiluminescence.

Protein Determination

Proteins were determined by the method of Lowry et al. [10] using BSA as standard.

Results

The incubation of rat liver microsomes in the presence of ascorbate-Fe^{+2} at 37 ° C for 180 minutes, resulted in peroxidation of membranes as evidenced by light emission (chemiluminescence). When comparing the control group with the groups in the presence of the SL extract, it was observed that at lower concentrations of the SL extract there was greater protection of the microsomal membranes, since the cpm were similar to the control group. (Figure 1).

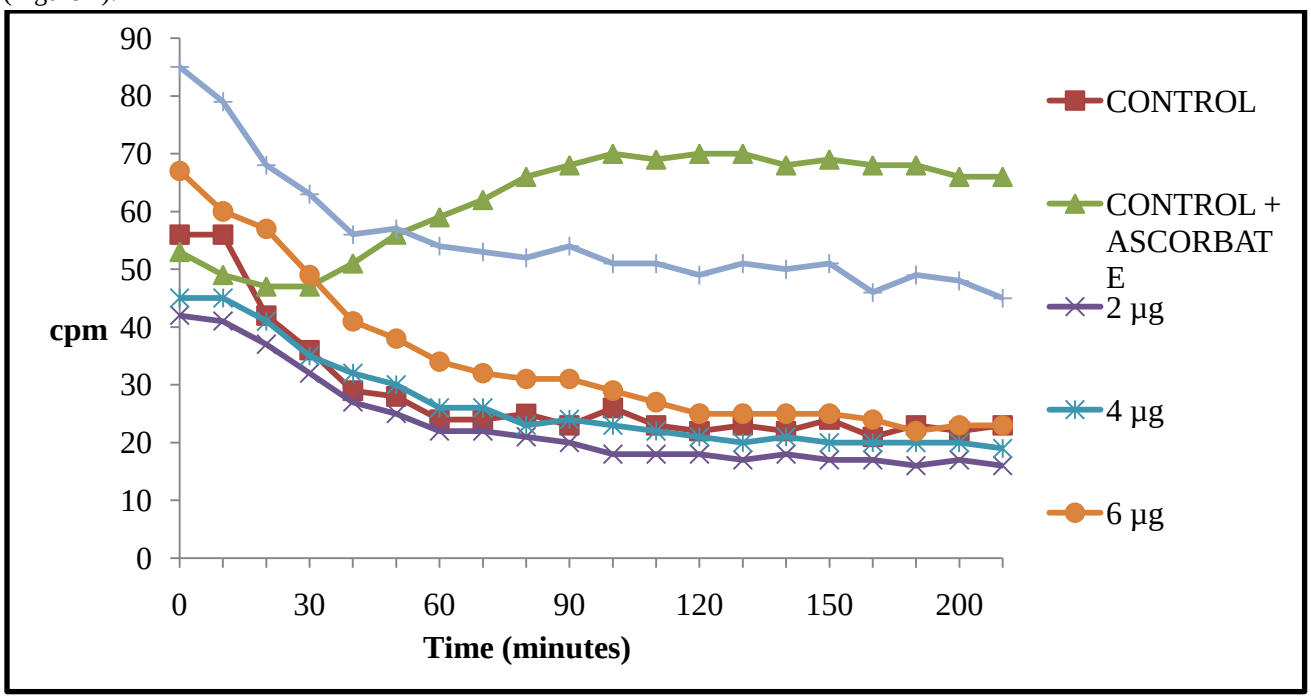

Table 1 shows the mean and its standard error of the averages of the cpm of the control sample 550.375 ± 95.2; of control + ascorbate 1375.5 ± 75.4 and of different concentrations used of SL extract.

Table 1: Light emission of rat liver microsomes during peroxidation (cpm) with different concentration of SL (2 µg, 4 µg, 6 µg and 8 µg). *

Microsomes	**Average ± ES**
Control	550,375 ± 95,2 [1]
Control + Ascorbate	1375,5 ± 75,4 [1,2,3,4,5]
2 µg	554,625 ± 45,2 [2,a]
4 µg	634 ± 77,8 [3,b]
6 µg	683,125 ± 35,9 [4,a,c]
8 µg	953,375 ± 92,6 [5,a,b,c]

* Data are given as the mean ±SD of experiments. Statistically significant differences in SL concentrations and microsomes liver rat control and peroxidized are indicated by [1,2,3,4] $p < 0{,}00001$; [5] $p < 0{,}001$ and [a,b,c,d] $p < 0.05$.

Discussion

The aim of our study was evaluate the capacity of tomato extract (lycopene) to protect liver microsomes against peroxidation. Rat liver microsomes incubated with tomato extract were protected against lipid peroxidation when compared to similar membranes from control group, as demonstrated by the results from chemiluminescence. In vitro lipid peroxidation studies are useful for the elucidation of possible mechanisms of peroxide formation in vivo [11, 12] since the composition of membranes causes susceptibility to peroxidative degradation [13]. Although considerable research has already been performed to characterize the changes in structure, composition and physical properties of membranes subjected to oxidation[14,15,16,17], it is important to know how biological compounds with antioxidant properties contribute to the protection of specialized membranes against deleterious effects produced by reactive oxygen species and other free radicals. Another studies were carried out to demonstrated that lycopene has antioxidative perspectives, which could prevent DNA damage and hence protect against mutations that cause cells cancer development [18]. Furthermore, recent studies reflect that lycopene may also modify molecular biomarkers of atherosclerosis [19] while studies on the dose-dependent effects of lycopene have reported conflicting results [3]. This conflicting results are in agree with our finding that show is possible pro oxidative activity doses dependent of lycopene.

Conclusion

It could be concluded that the use of lycopene generates protection from oxidative damage in the cell membranes of liver microsomes, functioning as an antioxidant. However, it should be clarified that using higher doses the lycopene generated less protection, but even so the effect was beneficial. More studies will be needed to continue investigating the antioxidant properties of lycopene in the peroxidation of biological membranes by evaluating different concentrations of them.

Acknowledgments

This work was supported by Secretaria de Ciencia y Técnica, Universidad Nacional de La Plata (grant11/V227).

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