Effect of lycopene from cooked tomatoes on serum antioxidant enzymes, lipid peroxidation rate and lipid profile in coronary heart disease

Bose K S C, Agrawal B K

ABSTRACT

Introduction: This present study aims to evaluate the beneficial effect of tomatoes, a rich source of lycopene, which is a relatively new carotenoid known to play an important role in human health and disease.

Methods: We investigated the lipid peroxidation rate by estimating malondialdehyde (MDA), levels of serum enzymes involved in antioxidant activities such as superoxide dismutase, glutathione peroxidase, glutathione reductase, reduced glutathione and lipid profile, which includes total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and very low density lipoprotein in a coronary heart disease (CHD) group and an age-matched control group.

Results: We observed significantly lower levels of serum antioxidant enzymes and very high lipid peroxidation rate in the CHD group, when compared to the controls (p-value is less than 0.001). At the same time, we observed significantly higher levels of lipids in the CHD group, when compared to the controls (p-value is less than 0.001). 60 days of tomato supplementation in the CHD group showed a significant improvement in the levels of serum enzymes involved in antioxidant activities and decreased lipid peroxidation rate (p-value is less than 0.001), but there were no significant changes in lipid profile (p-value is greater than 0.10).

Conclusion: These findings suggest that tomato lycopene may have considerable therapeutic potential as an antioxidant but may not be used as a hypolipidaemic agent in CHD.

Department of Biochemistry, People's College of Medical Sciences and Research Centre, Bypass Road, Bhanpur, Bhopal 462010, India

Bose KSC, PhD
Assistant Professor

Department of Biochemistry, Gandhi Medical College, Sultania Road, Bhopal 462001, India

Agrawal BK, PhD
Professor and Head

Correspondence to:
Dr K Subhash Chandra Bose
Tel: (91) 982 720 6260
Fax: (91) 755 400 5112
Email: subhashbose1@yahoo.co.in

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INTRODUCTION

According to Gupta and Gupta, 4.7 million people are suffering from coronary heart disease (CHD) in India.[1] The prevalence of CHD increased from 1.1% in 1960 to 9.7% in 1995 in urban populations. In rural areas, the prevalence increased from 2.0% in 1974 to 3.7% in 1995. According to the World Health Organisation (WHO), 3.8 million men and 3.4 million women worldwide die each year from CHD. The emphasis so far has been on the relationship between serum cholesterol levels and the risk of CHD. More recently, oxidative stress induced by reactive oxygen species (ROS) is also considered to play an important part in the aetiology of this disease. Oxidation of the circulating low-density lipoprotein [LDL (ox)] is thought to play a key role in the pathogenesis of atherosclerosis and CHD. According to this hypothesis, macrophages inside the arterial wall take up the LDL (ox) and initiate the process of plaque formation.[2] According to Cai and Harrison, increased ROS inactivates the production of nitric oxide ($NO^{\cdot}$), which accelerates the pathological phenomenon called endothelial dysfunction.[3] Alteration in endothelial function is an initial step in the pathogenesis of atherosclerosis.

Nutrition plays an important role in the development of these chronic diseases like CHD, hypertension and complications of diabetes mellitus. Diets rich in fruits and vegetables containing carotenoids have been of great interest because of their potential health benefits against these chronic diseases. Paralleling the growth wave is the evidence that oxidative-degraded free radical reactions are involved in CHD, beta-carotene, tocopherol and many other compounds have been investigated for many years for antioxidant properties. Dietary antioxidants such as vitamin E and beta-carotene have been shown in in vitro studies to prevent the formation of LDL(ox) and their

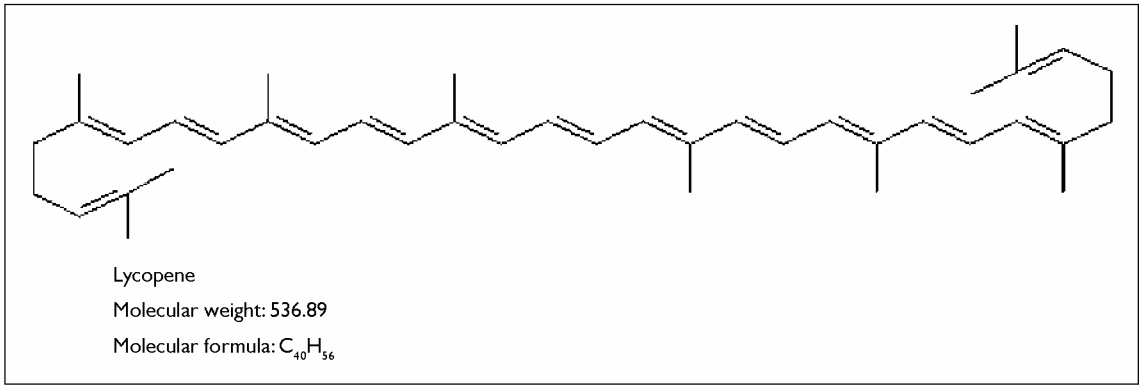

Fig. 1 Chemical structure of lycopene.[9]

uptake by macrophages. A number of epidemiological studies have shown an association between beta-carotene and the risk of cardiovascular diseases. Lycopene, a red pigment in tomatoes and tomato-based products, is an acyclic form of beta-carotene without pro-vitamin A activity. It has attracted substantial interest during recent times for its beneficial effect in reducing oxidative stress in CHD and other chronic diseases.[4-7] Its molecular weight is 536.89 and molecular formula is $C_{40}H_{56}$ with 89.45% carbon and 10.51% hydrogen. It is a highly-unsaturated hydrocarbon containing 11 conjugated and two unconjugated double bonds[8] (Fig. 1).[9]

Though lycopene is the most predominant carotenoid in human plasma, present naturally in greater amounts than β-carotene and other dietary carotenoids, which perhaps indicates its greater biological significance in the human antioxidant defence system,[10] and many epidemiological studies show the direct relationship between serum lycopene levels in CHD and other chronic diseases,[11-14] very few reports are available on experimental studies. In fact, no study has been done on the beneficial effect of long-term dietary supplementation of tomato lycopene in CHD. Therefore, the aim of this work was to study the antioxidant property of lycopene on ROS-induced oxidative stress and hypolipidaemic effect in CHD, and to see whether tomatoes can be recommended as one of the beneficial vegetables for CHD patients.

METHODS

50 healthy subjects, who were aged 35–55 years of either gender, were non-smokers and did not have any history of chronic systemic illness, were selected and treated as the control group. 30 CHD patients aged 35–55 years of either gender and in whom eletrocardiographical changes, such as increased VAT, increased R-wave amplitude, ST elevation that is sloped upwards, tall and widened T-waves, were observed, were selected from our medical outpatient department to study the beneficial effect of tomato lycopene. In both the control and CHD groups, the oxidative stress biomarkers, namely superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), and reduced glutathione (GSH) were estimated.[15-18] Lipid peroxidation rate was determined by estimating thiobarbituric acid reactive substances (TBARS),[19] and lipid profile included total cholesterol (TC), triglycerides (TG), and high density lipoproteins (HDL), using ready-made kits. Low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were calculated by the Friedewald equation. From the CHD group, 20 subjects were selected and advised to take 200 g of ripe tomatoes (cooked) every day for a period of 60 days. According to Agrawal and Rao, 100 g of tomatoes contain 12.70 mg of lycopene,[20] so we supplemented 200 g of tomatoes/day, as our aim was to supplement not less than 25 mg lycopene/day. During this period, CHD patients were not given any antioxidant and hypolipidaemic drugs as therapeutic measures. The above parameters were estimated at an interval of 15 days, which were compared before and after tomato supplementation in the CHD group at an interval of 15 days to study the antioxidant and hypolipidaemic effects of tomato lycopene in CHD (Table I).

For this study, a very simple method was adopted for cooking tomatoes. 200 g of ripe tomatoes (colour should be red) were weighed, chopped into small pieces, then one medium-size green chilli and salt were added for taste. This mixture was cooked for 8–10 minutes in 10 ml of soybean oil in a pressure cooker. This was served with *roti*. All the results were expressed as mean ± standard deviation. The Student t-test was used to assess statistical significance of the results between control and CHD groups before lycopene supplementation. p-value < 0.001 was considered as highly significant, p-value < 0.01 as significant, and p-value < 0.1 as insignificant. One-way ANOVA was applied to study the statistical significance of the difference in the mean values of the above said parameters observed in CHD patients as a result of tomato lycopene supplementation.

Table I. Descriptive characteristics of the two study groups (normal and CHD patients).

	35–40 years		41–45 years		46–50 years		51–55 years	
Control group (n = 50)	10 M	4 F	8 M	4 F	8 M	4 F	7 M	5 F
CHD group (n = 30)	6 M	1 F	6 M	1 F	5 M	3 F	5 M	3 F
CHD group for lycopene supplementation study (n = 20)	5 M	–	4 M	1 F	4 M	1F	3 M	2 F

M: male; F: female

Table II. Serum levels of enzymes involved in antioxidant activities, lipid peroxidation rate and lipid profile in CHD and control groups.

	Normal control	CHD group
Malondialdehyde (MDA) nmol/h	1.037 ± 0.213	2.062* ± 0.43
Super oxide dismutase (SOD) U/g Hb	1095.59 ± 140.71	481.51* ± 85.87
Glutathione peroxidase (GSH-Px) U/g Hb	82.04 ± 3.85	36.602* ± 11.63
Glutathione reductase (GR) U/L	63.052 ± 4.37	31.65* ± 8.84
Reduced glutathione (GSH) µmol/L	211.04 ± 15.27	109.84* ± 16.74
Total cholesterol (TC) mg/dL	174.87 ± 11.93	254.67* ± 26.15
Triglycerides (TG) mg/dL	97.37 ± 11.34	189.07* ± 18.80
High density lipoprotein (HDL) mg/dL	47.21 ± 3.50	33.58* ± 5.63
Low density lipoprotein (LDL) mg/dL	107.79 ± 10.68	186.32* ± 28.88
Very low density lipoproteins (VLDL) mg/dL	19.47 ± 0.26	37.81* ± 3.76

* $p < 0.001$ compared to normal control group.

Table III. One-way ANOVA of plasma MDA (nmol/h) level in CHD group at 0, 15, 30, 45 and 60 days of tomato lycopene supplementation.

Source of variation	Among days
Degree of freedom	04
Sum of squares	3.99
Mean sum of squares	0.99
F	5.08
p	< 0.001

Before tomato lycopene supplementation and at 15, 30, 45, 60 days of lycopene supplementation, the mean plasma MDA levels were 2.062, 1.989, 1.781, 1.547 and 1.623 nmol/h, respectively.

Table IV. One-way ANOVA of plasma SOD levels (U/g Hb) in CHD group at 0, 15, 30, 45 and 60 days of tomato lycopene supplementation.

Source of variation	Among days
Degree of freedom	04
Sum of squares	686,096.4
Mean sum of squares	171,524.1
F	38.52
p	< 0.001

Before tomato lycopene supplementation and at 15, 30, 45, 60 days of lycopene supplementation, the mean plasma SOD levels were 483.48, 494.25, 592.04, 670.55 and 676.70 U/g Hb, respectively.

Table V. One-way ANOVA of plasma GSH-Px level (U/g Hb) in CHD group at 0, 15, 30, 45 and 60 days of tomato lycopene supplementation.

Source of variation	Among days
Degree of freedom	04
Sum of squares	11,034.73
Mean sum of squares	2,758.68
F	47.94
p	< 0.001

Before tomato lycopene supplementation and at 15, 30, 45, 60 days of lycopene supplementation, the mean plasma GSH-Px levels were 36.19, 46.01, 58.96, 61.43 and 63.66 U/g Hb, respectively.

Table VI. One-way ANOVA of plasma GR level (U/L) level in CHD group at 0, 15, 30, 45 and 60 days of tomato lycopene supplementation.

Source of variation	Among days
Degree of freedom	04
Sum of squares	5,850.41
Mean sum of squares	1,462.60
F	36.07
p	< 0.001

Before tomato lycopene supplementation and at 15, 30, 45, 60 days of lycopene supplementation, the mean plasma GR levels were 31.12, 35.95, 46.09, 49.92 and 49.73 U/L, respectively.

RESULTS

The levels of oxidative stress biomarkers, namely SOD, GSH-Px, GR, GSH, lipid peroxidation activity (by estimating malondialdehyde [MDA]) and lipid profile which includes TC, TG, HDL, LDL, VLDL, in the CHD group were compared with the normal age-matched control group (Table II). Significantly higher levels of lipid peroxidation activity ($p < 0.001$), and very low

Table VII. One-way ANOVA of plasma GSH levels (µmol/L) in CHD group at 0, 15, 30, 45 and 60 days of tomato lycopene supplementation.

Source of variation	Among days
Degree of freedom	04
Sum of squares	68,607.85
Mean sum of squares	17,151.96
F	144.37
p	< 0.001

Before tomato lycopene supplementation and at 15, 30, 45, 60 days of lycopene supplementation, the mean plasma GSH levels were 105.08, 107.83, 157.31, 161.44 and 160.68 µmol/L, respectively.

Table VIII. One-way ANOVA of serum TC levels (mg/dL) in CHD group at 0, 15, 30, 45 and 60 days of tomato lycopene supplementation.

Source of variation	Among days
Degree of freedom	04
Sum of squares	105.08
Mean sum of squares	26.27
F	0.06
p	> 0.1

Before tomato lycopene supplementation and at 15, 30, 45, 60 days of lycopene supplementation, the mean serum TC levels were 254.95, 254.59, 253.88, 255.95 and 252.90 mg/dL, respectively.

Table IX. One-way ANOVA of serum TG levels (mg/dL) in CHD group at 0, 15, 30, 45 and 60 days of tomato lycopene supplementation.

Source of variation	Among days
Degree of freedom	04
Sum of squares	94.78
Mean sum of squares	23.69
F	0.10
p	> 0.1

Before tomato lycopene supplementation and at 15, 30, 45, 60 days of lycopene supplementation, the mean serum TG levels were 189.39, 187.01, 189.50, 189.22 and 188.85 mg/dL, respectively.

Table X. One-way ANOVA of serum HDL levels (mg/dL) in CHD group at 0, 15, 30, 45 and 60 days of tomato lycopene supplementation.

Source of variation	Among days
Degree of freedom	04
Sum of squares	0.5826
Mean sum of squares	0.1456
F	0.0001
p	> 0.1

Before tomato lycopene supplementation and at 15, 30, 45, 60 days of lycopene supplementation, the mean serum HDL levels were 33.11, 33.25, 33.04, 33.22 and 33.18 mg/dL, respectively.

Table XI. One-way ANOVA of serum LDL levels (mg/dL) in CHD group at 0, 15, 30, 45 and 60 days of tomato lycopene supplementation.

Source of variation	Among days
Degree of freedom	04
Sum of squares	200
Mean sum of squares	50.04
F	0.1
p	> 0.1

Before tomato lycopene supplementation and at 15, 30, 45, 60 days of lycopene supplementation, the mean serum LDL levels were 185.94, 183.93, 182.92, 184.89 and 181.92 mg/dL, respectively.

Table XII. One-way ANOVA of serum VLDL levels (mg/dl) in CHD group at 0, 15, 30, 45 and 60 days of tomato lycopene supplementation.

Source of variation	Among days
Degree of freedom	04
Sum of squares	3.79
Mean sum of squares	0.94
F	0.1
p	> 0.1

Before tomato lycopene supplementation and at 15, 30, 45, 60 days of lycopene supplementation, the mean serum VLDL levels were 37.94, 37.40, 37.90, 37.84 and 37.78 mg/dL, respectively.

levels of serum enzymes involved in antioxidant activities in CHD patients were observed, when compared with the controls. At the same time, significantly higher levels of lipid profile were observed in CHD patients, when compared with the control group ($p < 0.001$). The effect of tomato supplementation on oxidative stress biomarkers in CHD are shown in Tables III–VII. The mean plasma MDA level in the CHD group before lycopene supplementation was 2.062 nmol/hour, and the levels were decreased markedly to a minimum at 45 days of lycopene supplementation (Table III). The mean differences in MDA levels at different days of analysis during lycopene supplementation period were found to be significant ($p < 0.001$).

Before lycopene supplementation, the SOD levels in CHD group were found to be 481.51 U/g Hb. In this study, during lycopene supplementation in the CHD group, we observed a steady state improvement in the SOD levels which reached a maximum of 676.70 U/g Hb at 60 days (Table IV). The mean differences in SOD

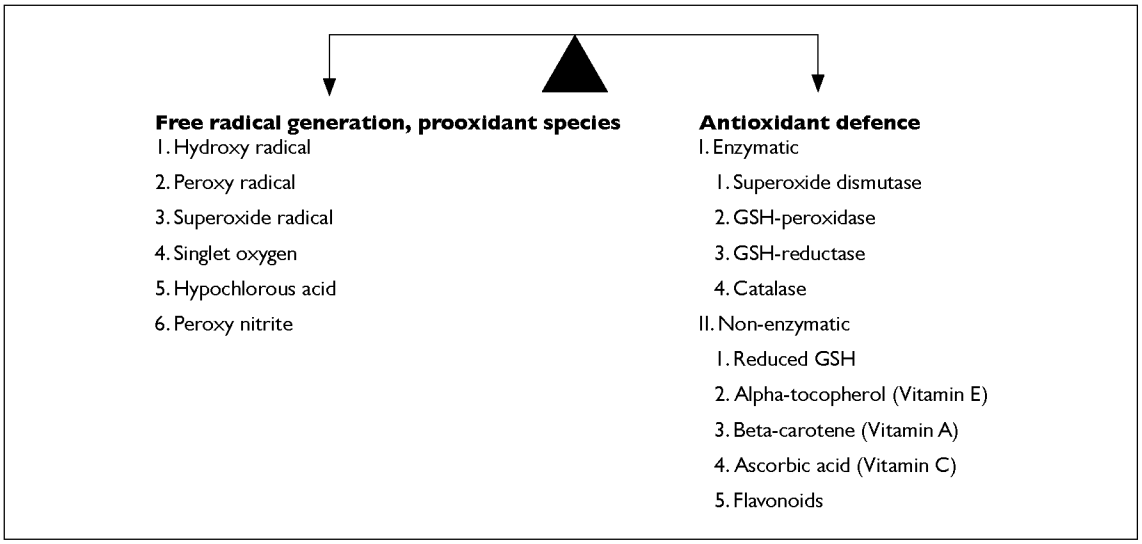

Fig. 2 Diagram shows the balance between free radical generation and antioxidant defence at the cellular level in the healthy state.

levels at different days of analysis during supplementation period were also found to be significant in the CHD group ($p < 0.001$). The same improvement in the levels of GSH-Px, GR and GSH activities were observed in the CHD group during lycopene supplementation (Tables V–VII), and the mean differences in the levels of above said parameters at different days of analysis during lycopene supplementation period were found to be significant ($p < 0.001$). But at the same time, we observed no significant changes ($p > 0.1$) in the lipid profile of CHD patients during lycopene supplementation period (Tables VIII–XII).

DISCUSSION

Several studies reported the beneficial effect of β-carotene intake in decreasing oxidative stress in diabetes mellitus. However, so far, no study has focused on the effect of long-term tomato lycopene supplementation on oxidative stress in CHD, where the singlet oxygen quenching ability of lycopene is twice as high as that of β-carotene and ten times higher than that of alpha tocopherol.[21] In this study, lower levels of oxidative stress biomarkers and increased lipid peroxidation of RBC membrane in the CHD group were observed, when compared with age-matched normal control, which indicates the increased oxidative stress in CHD, causing the imbalance between oxidants and antioxidants, which is normally maintained in healthy conditions (Fig. 2). Lycopene, having good free radical scavenging capacity because of its high number of conjugated double bonds, might have quenched the superoxide and other free radical anions which are highly released in CHD, thereby increasing the concentration of SOD, GSH-Px and GR, (the most important cytosolic enzymes involved in antioxidant activities), thereby reversing the disturbed balance to the antioxidant enzyme side, and decreasing oxidative stress. In this study, lycopene supplementation also increased the levels of GSH, the most important antioxidant metabolite that plays an important role in maintaining good levels of GSH-Px activity which is the main enzyme involved in removing the H_2O_2 generated from dismutation of superoxide anions by SOD. GSH is also the co-factor of several reducing enzymes such as dehydroascorbate reductase and endoperoxide isomerase.[22] The above results provide evidence that tomato lycopene also reduces the lipid peroxidation rate by acting as a good chain breaking antioxidant, which reacts with peroxy radicals formed in the propagation phase of lipid peroxidation to form carbon-centred radicals, which reacts readily and reversibly with oxygen to form new chain-carrying peroxyl radicals which are highly stable forms compared to ROS.

Fuhrman et al studied the effect of lycopene (10 μg) on macrophage cholesterol metabolism in comparison with cholesterol synthesis inhibitor fluvastatin (10 μg/ml).[23] In the J-774A.1 macrophage cell line, the cellular cholesterol synthesis from [^{3}H] acetate, but not from [C^{14}] mevalonate was suppressed by 73% by lycopene in comparison to 90% by fluvastatin, which indicates that lycopene inhibits HMGCoA reductase enzyme activity. In agreement with these in vitro observations, they studied the dietary supplementation with tomato lycopene (60 mg/day) in six healthy males for three months, which resulted in a significant 14% reduction in plasma LDL cholesterol concentration by inhibiting the HMGCoA reductase, which is a rate-limiting enzyme in cholesterol biosynthesis. In our study, no hypocholesterolaemic effect of lycopene was observed in CHD. This may be because it may not be so effective in inhibiting HMGCoA reductase

in a hyperlipidaemic environment, which persists in CHD patients.

Stahl and Sies showed that processing would increase the bioavailability of lycopene from tomatoes.[24] More significantly, the chemical form of lycopene is altered by high temperature, which makes it more easily absorbed by the body and also because lycopene is fat-soluble, absorption is improved when oil is added to the diet. Use of cooked tomatoes in the present study would have favoured absorption. In conclusion, the observations in this present study strongly prove the effective antioxidant property of tomato lycopene. Even though this effect is mainly due to lycopene, which accounts for 90% of total carotenoids and other phytochemicals present in tomatoes,[6] the participation of other carotenoids in the antioxidant effect of tomatoes cannot be ruled out. There is therefore a need for extensive study on other carotenoids, which are present in very low concentration in tomatoes.

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