Effect of Long Term Supplementation of Tomatoes (Cooked) on Levels of Antioxidant Enzymes, Lipid Peroxidation Rate, Lipid Profile and Glycated Haemoglobin in Type 2 Diabetes Mellitus

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ABSTRACT

The objective of the present study is to evaluate the beneficial effect of tomatoes, which are a rich source of lycopene, a relatively new carotenoid known to play an important role in human health. In this study, the lipid peroxidation rate was investigated by estimating malondialdehyde (TBARS) levels of antioxidant enzymes like SOD, GSH-Px, GR, GSH, lipid profile, which includes total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, very low density lipoprotein, and glycated haemoglobin HbA1c in (n = 40) the Type 2 diabetic group (n = 40) and an age-matched control group (n = 50). Significantly lower levels of antioxidant enzymes and very high lipid peroxidation rate in the Type 2 diabetic group were observed when compared to controls (p < 0.001). Likewise, significantly higher levels of lipid profile and glycated haemoglobin (HbA1c) in the diabetic group were observed when compared with control (p < 0.001). Long term tomato supplementation in diabetes mellitus showed a significant improvement in the levels of antioxidant enzymes and decreased lipid peroxidation rate (p < 0.001), but there were no significant changes in lipid profile and glycated haemoglobin HbA1c levels (p > 0.10). These findings suggest that tomato lycopene may have considerable therapeutic potential as an antioxidant but there was no significant lipid lowering effect in Type 2 diabetes mellitus.

Efectos de la Suplementación a Largo Plazo con Tomates (Cocidos) en los Niveles de Enzimas Antioxidantes, el Indice de Peroxidación Lipídica, el Perfil Lipídico, y la Hemoglobina Glicada en la Diabetes de Tipo 2

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RESUMEN

El objetivo del presente estudio es evaluar el efecto beneficioso del tomate como fuente rica en licopeno – un carotenoide relativamente nuevo, del cual se sabe que juega un importante papel en la salud humana. En este estudio, investigamos el índice de peroxidación lipídica, estimando los niveles MDA (TBARS) de las enzimas antioxidantes como SOD, GSH-Px, GR, GSH, el perfil lipídico, que incluye el colesterol total, los triglicéridos, los HDL, LDL, VLDL, y la hemoglobina glicada (HbA1c) en (n = 40) en el grupo diabético tipo 2 (n = 40) y el grupo de control pareado por edad (n = 50). En este estudio, observamos niveles significativamente más bajos de enzimas antioxidantes e índices de peroxidación lipídica muy altos en el grupo diabético tipo 2, en comparación con el grupo control (p < 0.001). Asimismo observamos niveles significativamente más altos de perfil lipídico y hemoglobina glicada (HbA1c) en el grupo diabético al comparársele con el grupo control (p < 0.001).

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INTRODUCTION

Diabetes is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity (1). Due to increasing obesity and altered dietary habits in both western and developing countries, the prevalence of Type 2 diabetes is growing at an exponential rate (2). In 2004, according to the World Health Organization (WHO), more than 150 million people worldwide suffered from diabetes. Its incidence is increasing rapidly, and it is estimated that by the year 2025, this number will double. The WHO has predicted that the major burden will occur in developing countries. There will be a 42% increase from 52

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to 72 million in developed countries and 170% increase from 84 to 228 million in the developing countries. The countries with the largest number of diabetic people are and will be, in the year 2025, India, China and the United States of America (USA).

Oxidative stress induced by reactive oxygen species (ROS) which are generated due to hyperglycaemia, is one of the major foci of recent research related to diabetes (3). There is strong evidence that the damage caused by ROS may play a significant role in causation of secondary symptoms in diabetes like neuropathy, nephropathy and retinopathy. Changes in oxidative stress biomarkers, including superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, glutathione levels, vitamins and lipid peroxidation are also observed in diabetes (4).

Antioxidants are protective agents that inactivate ROS and thereby significantly delay or prevent oxidative damage. Current dietary guidelines to combat chronic diseases including cancer, coronary heart disease (CHD) and diabetes recommended increased intake of plant products including fruits and vegetables which are rich in carotenoids as good sources of antioxidants (5, 6).

Table 1: Oxidative stress biomarkers in diabetic patients and normal age-matched controls

Parameters	Group-1 Normal control	Group-2 Diabetic patients
Malondialdehyde (MDA) nmol/h	1.037 ± 0.213	2.365 ± 0.403 $p < 0.001$
Super oxide dismutase (SOD) U/g Hb	1095.59 ± 140.71	417.79 ± 56.91 $p < 0.001$
Glutathione peroxidase (GSH-Px) U/g Hb	82.04 ± 3.85	26.06 ± 9.54 $p < 0.001$
Glutathione reductase (GR) U/L	63.052 ± 4.37	25.12 ± 4.41 $p < 0.001$
Reduced Glutathione (GSH) µmol/L	211.04 ± 15.27	93.49 ± 14.63
Total cholesterol (TC) mg/dl	174.87 ± 11.93	203.81 ± 9.53 $p < 0.001$
Triglycerides (TG) mg/dl	97.37 ± 11.34	167.40 ± 17.97 $p < 0.001$
High density lipoprotein (HDL) mg/dl	47.21 ± 3.50	40.04 ± 4.11 $p < 0.001$
Low density lipoprotein (LDL) mg/dl	107.79 ± 10.68	129.34 ± 11.21 $p < 0.001$
Very low density lipoproteins (VLDL) mg/dl	19.47 ± 2.26	33.48 ± 3.59 $p < 0.001$
Fasting blood glucose mg/dl	82.01 ± 12.48	164.35 ± 19.50 $p < 0.001$
Glycated haemoglobin % Hb	5.18 ± 0.43	7.39 ± 0.60 $p < 0.001$

The role of dietary antioxidants such as vitamin C, vitamin E, beta-carotenes in disease prevention has received much attention in recent years (7). The carotenoid compound called lycopene, which is in high levels in tomatoes and tomato-based food products is thought to play an important role in defence against chronic diseases like cancer and coronary heart diseases (8–12). Lycopene is a carotenoid compound, an acyclic isomer of beta-carotene and does not show any pro- vitamin A activity. It is a highly unsaturated hydrocarbon containing eleven conjugated and two unconjugated double bonds (13). It is the most predominant carotenoid in human plasma. This indicates its greater biological significance in human antioxidant defence system (14). Even though there are many epidemiological studies (15, 16) that indicate a direct beneficial relationship between serum lycopene level in diabetes and other chronic diseases, there is a paucity of data from experimental studies. In fact, no study has been done on the beneficial effects of long-term supplementation of lycopene in diabetics. The aim of this study is to investigate the antioxidant property of tomato lycopene on ROS induced oxidative stress and its lipid lowering effect in diabetes.

SUBJECTS AND METHODS

For this study, 50 healthy subjects between 35 to 55 years of age irrespective of gender and who were non-smokers and did not have any history of chronic systemic illness were randomly selected as normal controls. Forty Type 2 diabetic patients of a similar age group and matched for gender, who were non-smokers and did not have any history of diabetic complications like neuropathy, retinopathy, nephropathy and vascular symptoms were randomly selected from the outpatient clinic. In both control and diabetic groups, the authors estimated the oxidative stress biomarkers like SOD, GSH–Px, GR, reduced GSH by previously described methods (17–20). Lipid peroxidation rate was determined by estimating thiobarbituric acid reactive substances (TBARS) according to the method of Das *et al* (21). Lipid profile (total cholesterol, triglycerides, HDL by using ready-made kits, LDL, VLDL) was calculated by Friedewald equation, fasting blood sugar by using ready-made kits and glycated haemoglobin (HbA1c) by the method of Sheela *et al* (22). Then from the diabetic group, 30 diabetic subjects were randomly selected and were advised to take 200g of ripe tomatoes (cooked) everyday for a period of 60 days. In this period, diabetic subjects were not given any antioxidant in therapeutic measure. The above said parameters were estimated and were compared between the diabetic group before tomato supplementation and the diabetic group after supplementation at an interval of 15 days to determine the antioxidant and hypolipidaemic effect of tomato lycopene in diabetes. All the results were expressed as mean ± SD. Student's *t*-test was used to assess statistical significance of the results.

RESULTS

The levels of oxidative stress biomarkers like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GR), reduced glutathione (GSH), lipid peroxidation rate (by estimating malondialdehyde MDA), TC, TG, HDL, LDL, VLDL, fasting blood glucose, HbA1c in the diabetic group were compared with a normal age matched control group (Table 1). There were significantly higher levels of lipid peroxidation rate ($p < 0.001$) and very low levels of antioxidant enzymes in diabetes when compared with control. There were significantly higher levels of lipid profile, fasting blood glucose and HbA1c in diabetic patients when compared with the control group ($p < 0.001$).

Table 2 shows the effect of tomato supplementation on oxidative stress in diabetes. After 15 days of tomato supplementation (200g/day), though there were no significant improvement in the lipid peroxidation rate and SOD levels ($p > 0.1$), there was a statistically significant improvement ($p < 0.001$) in the levels of GSH (reduced), and GR (glutathione reductase).

There was an overall increase in the levels of SOD, GSH-Px, GR and GSH ($p < 0.001$) and statistically signifi-

Table 2: Tomato supplementation on oxidative stress in diabetics

Parameters	Group-2 Diabetic patients	15 days of tomato supplement	30 days of tomato supplement	45 days of tomato supplement	60 days of tomato supplement
Malondialdehyde (MDA) nmol/h	2.365 ± 0.403 $p > 0.1$	2.283 ± 0.403 $p < 0.001$	2.015 ± 0.318 $p < 0.001$	1.780 ± 0.399 $p < 0.001$	1.747 ± 0.360
Super oxide dismutase (SOD) U/g. Hb	417.79 ± 56.91	436.85 ± 37.21 $p > 0.1$	596.20 ± 39.57 $p < 0.001$	657.85 ± 34.87 $p < 0.001$	659.53 ± 35.85 $p < 0.001$
Glutathione peroxidase (GSH-Px) U/g. Hb	26.06 ± 9.54	± 33.78 ± 5.41 $p < 0.01$	51.95 ± 3.98 $p < 0.001$	55.96 ± 4.94 $p < 0.001$	56.08 ± 4.82 $p < 0.001$
Glutathione reductase (GR) U/L± 4.41	25.12 $p < 0.001$	30.43 ± 4.70 $p < 0.001$	39.98 ± 3.07 $p < 0.001$	47.04 ± 4.14 $p < 0.001$	47.95 ± 3.99
Reduced Glutathione (GSH) µmol/L	93.49 ± 14.63	101.98 ± 12.79 $p < 0.001$ $p < 0.001$	126.90 ± 14.89 $p < 0.001$	154.30 ± 9.79 $p < 0.001$	158.29 ± 9.57
Total cholesterol (TC) mg/dl	203.81 ± 9.53 $p < 0.1$	202.79 ± 10.42 $p < 0.1$	192.47 ± 5.64 $p < 0.1$	192.35 ± 8.13 $p < 0.1$	191.14 ± 8.19
Triglycerides (TG) mg/dl	167.40 17.97 $p < 0.1$	166.09 ± 14.41 $p < 0.1$	167.04 ± 13.44 $p < 0.1$	167.46 ± 13.23 $p < 0.1$	163.83 ± 11.08
High density lipoprotein (HDL) mg/dl	40.04 ± 4.11	40.94 ± 3.70 $p < 0.1$	40.87 ± 2.79 $p < 0.1$	40.59 ± 3.42 $p < 0.1$	40.21 ± 3.37 $p < 0.1$
Low density lipoprotein (LDL) mg/dl	129.34 11.21	128.62 ± 11.88 $p < 0.1$	119.52 ± 6.40 $p < 0.1$	118.24 ± 8.34 $p < 0.1$	118.15 ± 8.65 $p < 0.1$
Very low density lipoproteins (VLDL) mg/dl	33.48 ± 3.59	33.21 ± 2.88 $p < 0.1$	33.40 ± 2.68 $p < 0.1$	33.48 ± 2.65 $p < 0.1$	32.76 ± 2.27 $p < 0.1$
Fasting blood glucose mg/dl	161.64 164.35 ± 19.50 $p < 0.1$	162.69 ± 9.72 $p < 0.1$	161.27 ± 9.88 $p < 0.1$	161.10 ± 9.32 $p < 0.1$	± 8.67
Glycated haemoglobin % Hb	7.39 ± 0.60	7.32 ± 0.50 $p < 0.1$	7.26 ± 0.60 $p < 0.1$	7.20 ± 0.63 $p < 0.1$	7.15 ± 0.62 $p < 0.1$

cant decrease in lipid peroxidation ($p < 0.001$) at 30 days of tomato supplementation. The raised antioxidant enzyme levels and steep decline in lipid peroxidation rate was observed up to 45 days of tomato supplementation. There were no significant changes in the levels of the above said parameters after 45 days of tomato supplementation.

No significant changes in lipid profile, fasting blood sugar and HbA1c were observed from day one to day 60 of tomato supplementation ($p > 0.10$).

DISCUSSION

Several studies reported the beneficial effect of beta carotene intake in decreasing oxidative stress in diabetes, however, the authors are unaware of studies that focussed on the effect of tomato lycopene on oxidative stress in diabetes. In lycopene, the singlet oxygen quenching ability is twice as high as that of beta carotene and 10 times higher than that of alpha tocopherol (23). In this study, there was mean lower levels of oxidative stress biomarkers and increased lipid peroxidation of RBC membrane in the diabetic group when compared with age-matched normal control, which indicates the increased oxidative stress in diabetes, causing the imbalance between oxidants and antioxidants, which is normally maintained in healthy conditions, a key factor for diabetic complications. Lycopene, having good free radical scavenging capacity because of its unique structure (high number of conjugated double bonds) might have quenched the superoxide and other free radical anions which are released in diabetes due to abnormal metabolism, thereby increasing the concentration of SOD, GSH-Px, GR, the most important cytosolic antioxidant enzymes, thereby reversing the disturbed balance to the antioxidant enzyme side, and causing decreased oxidative stress.

In this study, lycopene supplementation also increased the levels of reduced glutathione, the most important antioxidant metabolite that plays an important role in maintaining good levels of glutathione peroxidase (GSH-Px) activity. This is the main enzyme involved in removing the H_2O_2 generated from dismutation of superoxide anions by superoxide dismutase (SOD). GSH is also the co-factor of several reducing enzymes such as dehydro-ascorbate reductase and endoperoxide isomerase (24). The above results suggest that tomato lycopene also reduces 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. These radicals then react readily and reversibly with oxygen to form new chain-carrying peroxyl radicals which are stabler than ROS.

Fuhrman *et al* (25), in there small supplementation study, observed significant reduction in plasma LDL cholesterol levels in healthy subjects by inhibiting the HMGCoA reductase which is a rate-limiting enzyme in cholesterol biosynthesis. In the present study, the authors did not observed any hypocholesteroleamic effect of lycopene in diabetes. This may be so because of increased availability of acetylCoA, a positive regulator of HMG CoA reductase, which is produced by increased beta oxidation of fatty acids in diabetes. At the same time, no significant changes in fasting blood sugar and HbA1c levels in diabetes were observed. This suggests no role of lycopene on glucose uptake, metabolism of glucose by extra hepatic tissue and non-enzymatic glycation of haemoglobin in diabetes.

Stahl and Sies (26) showed that processing will increase the bioavailability of lycopene from tomatoes. More significantly, the chemical form of lycopene is altered by temperature, and this makes it more easily absorbed by the body. Also lycopene is fat soluble and this absorption is improved when oil is added to the diet, hence the use of cooked tomatoes in this study.

The above observations support the effective antioxidant property of tomato lycopene. Even though this effect is mainly due to the lycopene, which accounts for 90% of total carotenoids and other phytochemicals present in tomatoes (11), the participation of other carotenoids in the antioxidant effect of tomatoes cannot be ruled out. So there is a need for extensive study on other carotenoids which are present in low concentration in tomatoes.

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