EFFECT OF SHORT TERM SUPPLEMENTATION OF TOMATOES ON ANTIOXIDANT ENZYMES AND LIPID PEROXIDATION IN TYPE –II DIABETES

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ABSTRACT

The objective of the present study is to evaluate the beneficial effect of tomatoes, which are rich source of Lycopene, relatively a new carotenoid known to play an important role in human health and disease. In this study lipid peroxidation rate was measured by estimating Malondialdehyde and the levels of serum enzymes involved in antioxidant activities like Super Oxide Dismutase, Glutathione Peroxidase, Glutathione Reductase, Reduced Glutathione, in type-II diabetic group (n = 40) and age matched control group (n = 50), and observed significantly lower levels of antioxidant enzymes and very high lipid peroxidation rate in type-II diabetes when compared to control group ($p < 0.001$). Short term supplementation with tomatoes (cooked) to diabetic group for a period of 30 days, showed a significant improvement in antioxidant enzyme levels ($p < 0.001$) and decreased lipid peroxidation rate ($p < 0.001$) suggesting the supplementation with tomato lycopene may serve as the best method of preventing the oxidative stress in diabetic patients.

KEY WORDS

Tomato lycopene, Malondialdehyde, Super Oxide Dismutase, Catalase, Reduced Glutathione, Glutathione Reductase, Glutathione Peroxidase.

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 II diabetes is growing at an exponential rate (2). In 2004, according to the World Health Organization more than 150 million people worldwide suffer from diabetes. The WHO has predicted that the major burden will occur in developing countries. The countries with the largest number of diabetic people will be, India, China and United States in the year 2025.

Oxidative stress induced by reactive oxygen species (ROS), which are generated due to hyperglycemia, 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 etc. Changes in oxidative stress biomarkers, including Superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GR), Glutathione peroxidase (GSH-Px), Glutathione levels (GSH), vitamins and lipid peroxidation are also observed in diabetes (4).

Antioxidants are protective agents that inactivate Reactive Oxygen Species (ROS) and thereby significantly delay or prevent oxidative damage. The role of dietary antioxidants including Vitamin-C, Vitamin-E and β -carotenes in disease prevention has received much attention in recent years (5) and much work has been done on them. Very recently a new carotenoid compound called Lycopene, a red pigment which is rich in tomatoes and tomato based food products which is thought to play an important role in defense against chronic diseases like cancer, coronary heart diseases has been discovered (6-9) Lycopene is a carotenoid compound, an acyclic isomer of β -carotene and does not show any pro vitamin A activity. It is a highly unsaturated hydrocarbon containing

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eleven conjugated and two unconjugated double bonds. It is the most predominant carotenoid in human plasma present naturally in greater amount than β -carotene and other dietary carotenoids. This perhaps indicates its greater biological significance in human antioxidant defense system (10). Even though many epidemiological studies (11,12) prove the direct relationship between serum lycopene level in diabetes and other chronic diseases, very few volumes are available on supplementation studies. In fact, no study has been done on the beneficial effects in diabetes with long-term or short-term supplementation of lycopene. Hence the aim of this work was to study the antioxidant property of tomato lycopene on ROS induced oxidative stress in diabetes, so that tomatoes can be recommended as one of the beneficial vegetables for diabetes.

MATERIALS AND METHODS

Fifty healthy subjects of age group between 35 to 55 years irrespective of sex who were non-smokers with no history of any chronic systemic illness were selected and treated as normal control group. 40 type II diabetic patients of age group between 35 to 55 years irrespective of sex who were non-smokers with no history of diabetic complication like neuropathy, retinopathy, nephropathy and vascular symptom were selected from out patient department (OPD) medicine to study the beneficial effect of tomatoes (cooked). In both control and diabetic group the oxidative stress biomarkers namely SOD, GSH-Px, GR, and reduced GSH were estimated (13-16). Lipid peroxidation rate was determined by estimating thiobarbituric acid reactive substances (TBARS) (17). From the diabetic group thirty subjects were selected and advised to take 200g of ripe tomatoes (cooked) everyday for a period of 30 days. In this period diabetic subjects were not given any antioxidant as therapeutic measure. The above said parameters were estimated at an interval of 15 days during the tomato (cooked) supplementation period, and were compared with the levels of above said parameters before the supplementation of tomatoes, to study the antioxidant effect of tomato lycopene in diabetes.

For this study very simple method was adopted in cooking tomatoes. 200 grams of ripe tomatoes (colour should be red) were weighed, chopped into small pieces then added one medium size green chilly and salt for taste. This mixture is cooked for 8-10 minutes in 10ml of soybean oil in a pressure cooker. Served this with roti.

All the results were expressed as Mean $\pm$ SD. Student's 't' test was used to assess statistical significance of the results between control and diabetic group before tomato lycopene

supplementation. And the p values <0.001 were 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 diabetic patients during tomato lycopene supplementation period.

RESULTS AND DISCUSSION

The levels of oxidative stress biomarkers namely SOD, GSH-Px, GR, GSH and Lipid peroxidation rate by estimating malondialdehyde (MDA) in diabetic group were compared with normal age matched control group (Table-I). Significantly higher levels of lipid peroxidation rate (p <0.001), and very low levels of serum enzymes involved in antioxidant activities in diabetes were observed when compared with control (p <0.001).

Table 1 : Serum enzymes involved in antioxidant activities, lipid peroxidation in type-II diabetes in comparison with normal control group.

Parameters	Group-1 Normal control	Group-2 Diabetic patients
MDA nmol/h	1.037 $\pm$ 0.213	2.365*** $\pm$ 0.403
SOD U/g. Hb	1095.59 $\pm$ 140.71	417.79*** $\pm$ 56.91
GSH-Px U/g. Hb	82.04 $\pm$ 3.85	26.06*** $\pm$ 9.54
GR U/L	63.052 $\pm$ 4.37	25.12*** $\pm$ 4.41
GSH μ mol/L	211.04 $\pm$ 15.27	93.49*** $\pm$ 14.63

*** p < 0.001

Table-2 shows the effect of tomato supplementation on Oxidative Stress in diabetes. The mean plasma MDA levels in diabetic group before lycopene supplementation was found to be 2.36 nmol/h. the levels were decreased markedly to minimum at 30 days of lycopene supplementation. 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 diabetic group were found to be 417.7 U/g Hb. The improvement in SOD levels was observed during lycopene supplementation period. The mean differences in SOD levels at different days of analysis during lycopene supplementation period were found to be significant (p <0.001). The same improvement in the levels of GSH-Px, GR and GSH activities were observed during lycopene supplementation, and the mean differences in the levels of above said parameters at different days of

Table 2 : Antioxidant enzymes and lipid peroxidation in Diabetic Group at 0, 15 and 30 days of tomato Lycopene supplementation.

Parameters	Before tomato Supplement	15 days of tomato supplement	30 days of tomato supplement	One-way ANOVA			
				DF	SS	MS	F
MDA nmol/h	2.36 ± 0.39	2.28 ± 0.40	2.01 ± 0.31	02	1.99	0.99	7.61*
SOD U/g. Hb	417.79 ±57.28	436.85 ±37.21	596.20 ±39.57	02	575778.95	287889.47	92.52*
GSH-Px U/g. Hb	26.06 ±10.09	33.78 ±5.41	51.95 ±3.98	02	10267.25	5133.62	90.36*
GR U/L	25.12 ±3.81	30.43 ±4.70	39.98 ±3.07	02	3449.3	1724.65	23.21*
GSH µmol/L	93.49 ±14.22	101.98 ±12.79	126.9 ±14.89	02	10267.25	5133.62	90.36*

DF- Degree of Freedom Between Groups

SS- Sum of Square Between Groups

MS- Mean Sum of Square Between Groups

*P <0.001

analysis during lycopene supplementation period were found to be significant ($p < 0.001$). (Table 2).

Several studies reported the beneficial effect of β -carotene intake in decreasing oxidative stress in diabetes. However so far no studies have focused on the effect of tomato lycopene on oxidative stress in diabetes whose singlet oxygen quenching ability is twice as higher than that of β -carotene and 10 times higher than that of alpha tocopherol (18). In this study lower levels of oxidative stress biomarkers and increased lipid peroxidation of RBC membrane in diabetic group were observed when compared with age matched normal control, which straightaway 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. The 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 highly released in diabetes due to the abnormal metabolism, 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, thus responsible for 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 activity which is the main enzyme involved in removing the H_2O_2 generated from dismutation of superoxide anions by Super Oxide Dismutase. GSH is also the co-factor of several reducing enzymes such as dehydroascorbate reductase and endoperoxide isomerase (19). From the above results it is proved that tomato lycopene also reduces the lipid peroxidation rate by acting as a good chain breaking antioxidant, which reacts with peroxy radicals formed in propagation phase of lipid peroxidation to form

carbon centered radical, which reacts readily and reversibly with oxygen to form a new chain – carrying peroxy radicals which are highly stable forms than ROS.

Stahl (20) showed that processing will increase the bioavailability of lycopene from tomatoes. More significantly, the chemical form of lycopene is altered by the 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.

The observations in the present study strongly prove the effective antioxidant property of tomato lycopene. Even though this effect is mainly due to lycopene, which accounts for ninety percent of total carotenoids and other phytochemicals present in tomatoes (8), 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 very low concentration in tomatoes.

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