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## To Study the Effect of Raw and Processed Tomato Supplementation on Plasma Total Antioxidant Capacity

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### Research Article

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#### ABSTRACT

Tomatoes and its products are good source of antioxidant like vitamin C, vitamin E,  $\beta$ -carotene, phenolic acids, flavonoids and lycopene. The bioavailability of these components is influenced by various factors including processing technology. This investigation was aimed to study the effect of raw and processed tomato supplementation on plasma total antioxidant capacity. For this study, 30 healthy female subjects (aged 21-23 years) were selected and divided into three equal groups. The subjects of groups II and III were supplemented with raw and processed tomatoes respectively for 30 days whereas group I was considered as control group. Their fasting blood samples were collected and analyzed for plasma vitamins C, A and E, Plasma and RBC glutathione, total antioxidant capacity and lipid profile, before and after tomato supplementation. Plasma vitamin C and RBC glutathione were increased significantly in group II and III. Plasma vitamin A was significantly increased in group II whereas plasma vitamin E was significantly increased in group III. Plasma total antioxidant capacity did not show significant change in group II and III. Thus, the daily supplementation of raw or processed tomato helps in maintaining the normal plasma antioxidant status and helps in preventing the chronic diseases.

#### INTRODUCTION

A high consumption of fruit and vegetables, in the diet, contributes to an increased intake of key nutrients, such as vitamins, minerals, antioxidant compounds and dietary fibre, with subsequent beneficial effects on health. Many epidemiological studies indicated that the frequent consumption of fruits and vegetables can significantly reduce the prevalence of chronic diseases<sup>[1]</sup>. Fruits and vegetables are rich source of natural antioxidants, such as vitamins and phenolic compounds which have protective effect against many diseases such as cancer cardiovascular disease (CVD), metabolic disorders and stroke<sup>[2,3]</sup>. The potential disease prevention of fruits and vegetables are because of their role in antioxidant capacity, modulation of detoxification enzyme, stimulation of immune system, decrease in platelet aggregation, and alteration in cholesterol metabolism, modulation of steroid hormone concentration and hormone metabolism, blood pressure reduction and antibacterial and antiviral activity<sup>[4]</sup>. Because of these advantages of natural antioxidants, increasing awareness has been paid to screen out preferably natural sources of antioxidants<sup>[5]</sup>.

Among all the fruits and vegetables, tomatoes (*Lycopersicon esculentum*) and its products such as puree, paste and sauce are the integral part of the diet worldwide and fourth most commonly consumed vegetable<sup>[6]</sup>. Tomatoes are the most commonly grown vegetable all around the world. Tomatoes are good source of vitamins like  $\beta$ -carotene, vitamin-C, vitamin-E, phenolic acid like ferulic acid, chlorogenic acid and caffeic acid, flavanoids like quercetin, minerals like copper, manganese and zinc and rich source of lycopene<sup>[7]</sup>. Numerous studies suggested that tomato consumption reduces the risk of chronic diseases such as

cardiovascular disease and cancer<sup>[8]</sup>. This defensive action is typically accredited to the tomato antioxidant lycopene which is red pigmented carotene compound. Along with lycopene, other antioxidants like vitamin C, vitamin E, polyphenol and flavonoids provide additional antioxidant capacity to tomatoes<sup>[9]</sup>.

The lycopene content of tomato depends on variety, maturity stage, environmental factors, cultural practices and agronomic aspects<sup>[7]</sup>. The quenching ability of lycopene is two times higher than  $\beta$ -carotene and ten times higher than  $\alpha$ -tocopherol. Growing evidence from epidemiological studies has indicated that lycopene is the major carotenoid in tomato recognized for its antioxidant activities on the cellular level, might be more significant than other carotenoids in preventing atherosclerosis and cardiovascular diseases<sup>[10,11]</sup>. Regular consumption of tomato and its products can even reduce oxidative DNA damage<sup>[12]</sup>. Furthermore, the bioavailability of lycopene is highly variable depending on food matrix and processing and the form in which it is consumed<sup>[13]</sup>. Thus, the objective of the present study was to study the effect of raw and processed tomato supplementation on plasma total antioxidant capacity (TAC).

## MATERIALS AND METHODS

### Study population and design

For this investigation, 30 young healthy female volunteers aged between 21-23 years were purposively recruited. Their interview was conducted for the absence of any metabolic disorder or chronic disease. All were free from CVD, hypertension, diabetes mellitus, dyslipidemia, or family history of premature vascular disease. Selected 30 female were divided into three different groups, consisting 10 females in each group. The anthropometric parameters of the recruited subjects were studied at the beginning of the study. Height (m) and weight (kg) were estimated using standard scale and weighing machine respectively and BMI [weight (kg)/Height (m<sup>2</sup>)] was calculated. All the subjects were recommended not to change their dietary schedule but requested not to take any vitamin supplements during the experimental period. Before the supplementation period starts, all the subjects were asked to have tomato free diet period for 15 days and that was considered as “wash out period”. On the 16<sup>th</sup> day, actual experimental period was started with the collection of fasting blood sample. Collected blood samples were analyzed for different biochemical parameters. On the same day (16<sup>th</sup> day), tomato supplementation (150 gm) was started along with a slice of brown bread (Kalory, approximately 18 gm) and 5 gm butter (Amul) and continued for 30 days. From that, group-I were considered as control group and subjects of group II and III were given raw and processed tomato supplementation respectively. On 31<sup>st</sup> day of supplementation, fasting blood samples were collected and estimated for different biochemical parameters. Obtained data were analyzed statistically.

### Raw and processed tomato supplementation

During this period, locally available tomatoes (based on even ripeness and freshness), bread (Kalory) and butter (Amul) were procured from market and supplemented to different groups (**Table 1**). Tomatoes were washed with clean water and cut into even pieces. Exactly, 150 gm of tomatoes were weighed in bowl and pinch of salt was added. The subjects of group-II, were given that with one slice of bread (approximately 18 gm) and 5 gm of butter for 30 days. Similarly, the subjects of group-III were supplemented with tomato soup, prepared out of 150 gm of tomatoes along with one slice of bread (approximately 18 gm) and 5 gm of butter for 30 days. For the preparation of tomato soup, 1.500 kg of tomatoes was washed, cut and pressure cooked for 10 minutes. Pressure cooked tomatoes were blended for five minutes with hand blender (Boss company) and salt was added. Prepared soup was distributed equally in ten bowls with skin and seeds and immediately served to the subjects of group-III. Subjects of control groups were fed with a slice of brown bread and 5 gm butter for 30 days.

**Table 1.** “Tomato supplementation in different groups.”

Groups	Supplementation		
	Tomato (150 gm)	Brown Bread (Kalory)	Butter (Amul)
I	-	1 slice	5gm
II	Raw tomato	1 slice	5gm
III	Tomato soup	1 slice	5gm

### Analysis of tomato

The selected raw tomatoes, used during experimental period were analyzed once in a week in duplicate for their vitamin C, lycopene and total antioxidant capacity. Vitamin C was estimated using titrimetric method described by Rangana (1979), lycopene was estimated using modified method described by Veazie et al., (2002) and total antioxidant capacity (TAC), as per the method given by Banzie and Strain (1996)<sup>[14,15,16]</sup>.

### Collection of blood samples and biochemical analysis

Fasting blood samples were collected before (after 15 days of wash out period) and after (on 31<sup>st</sup> day) supplementation period. With the help of trained lab technician, approximately 5 ml of venous blood sample was collected from each subject in clean and dried centrifuge tube, containing dried *ethylene diamine tetra acitic acid* (EDTA). Collected blood samples were centrifuged (Remi Research Centrifuge) to obtain plasma. Collected plasma was stored in labeled plastic vials (ependrof) and

stored at  $-20^{\circ}\text{C}$ . Plasma vitamin C was performed by the method given by Roe and Kuether (1943) and Bessay et al, (1947)<sup>[17,18]</sup>. Plasma vitamin A was estimated by the method given by Neeld and Pearson (1963)<sup>[19]</sup>. For the estimating plasma vitamin E, modified method given by Desai (1984) was implemented and for estimating total antioxidant capacity (TAC), method given by Banzie and Strain (1996) was followed<sup>[16,20]</sup>.

After separating plasma, the content was washed with saline solution for 2 to 3 times to obtained RBCs. RBCs were diluted with 3 ml of distilled water and stored at  $-20^{\circ}\text{C}$  and analyzed for status of RBC glutathione (RBC-GSH) with the method given by Ellman (1959)<sup>[21]</sup>.

### Statistical analysis

The results were expressed as mean  $\pm$  standard error of mean (SEM). Paired t-test was performed and the difference between the variables were examined for significance by using one way analysis of variance procedure and Duncan with the level of significance of  $p \leq 0.05$  in SPSS version 10.0 (Statistical Package for Social Sciences).

## RESULTS AND DISCUSSION

### Anthropometric measurements

The anthropometric characteristics are presented in **Table 2**, which shows the mean value of height was same in all the groups (1.155 m). The body weight ranged from 45.39 to 48.00 kg. Using the data of height and weight BMI was calculated and found almost equal in all the three groups i.e.19.22 to 20.05  $\text{kg}/\text{m}^2$ .

**Table 2.** "Height, Weight and Body mass index (BMI) of the subjects in different groups before the experiment."

Groups	Height (m)	Weight (kg)	BMI ( $\text{kg}/\text{m}^2$ )
I	1.556 $\pm 0.01$	48.00 $\pm 1.30$	19.98 $\pm 0.38$
II	1.552 $\pm 0.01$	46.76 $\pm 0.92$	20.05 $\pm 0.26$
III	1.553 $\pm 0.01$	45.39 $\pm 1.09$	19.22 $\pm 0.34$

Values are the mean  $\pm$  SEM

### Tomato analysis

Tomatoes that are used during experimental period were analyzed once in a week in duplicate for their vitamin C, lycopene and total antioxidant capacity (**Table 3**).

**Table 3.** "Vitamin C, lycopene and total antioxidant capacity (TAC) of tomato."

Parameters	Analytical value
Vitamin C (mg %)	21.00 $\pm 0.74$
Lycopene (mg/kg)	84.15 $\pm 6.22$
TAC ( $\mu\text{g TE}/\text{g}$ )	5194.93 $\pm 162.96$
TAC ( $\mu\text{g AAE}/\text{g}$ )	$\pm 137.90$

Values are the means of eight determinants  $\pm$  SEM

TE: Trolox equivalent

AAE: Ascorbic acid equivalent

Tomatoes are rich source of vitamin C which acts as an antioxidant by neutralizing the action of free radicals in the biological systems. The obtained values of vitamin C (21mg %) found was similar to the reported values (20 mg %) by Ilahy et al., (2011) and Juroszek et al., (2009)<sup>[22,23]</sup>. An antioxidant, lycopene is fat soluble pigment belongs to carotenoids family. Dietary intake of lycopene containing food has been shown to decreased risk of chronic diseases, such as cancer and cardiovascular disease<sup>[24]</sup>. The mean value of lycopene was found 81.15 mg/kg of tomatoes (8.115 mg/100 gm). Ilahy et al., (2011) have reported the lycopene content less than 10 mg/ 100 gm in different Italian varieties of tomato<sup>[22]</sup>. The mean values of total antioxidant capacity were found 5194.93 and 4396.27  $\mu\text{g}$  equivalent to trolox and ascorbic acid / g respectively from experimental tomatoes. The mean values of total antioxidant capacity were found 5194.93 and 4396.27  $\mu\text{g}$  equivalent to trolox and ascorbic acid / g respectively from experimental tomatoes.

### Biochemical analysis

The observed value of vitamin C in plasma was 1.10, 0.96 and 1.08 mg % (**Table 4**) in group I, group II and group III respectively before supplementation which shows no significant difference. After the supplementation it was increased 18.18 %, 112.50 % and 46 % in group I, II and III respectively. Increased plasma vitamin C in the subjects of group I had no any relation

with the supplementation as it is control group. The resultant cause of increased level in plasma vitamin C in this group may be due to their routine diet. Subjects of Group II showed maximum increment in plasma vitamin C who were supplemented with raw tomatoes, which revealed that the supplementation of raw tomato showed better response for increasing plasma vitamin C than the processed tomato. This is because of loss of vitamin C during thermal processing<sup>[25]</sup>. Garcia-Alonso et al (2012) have also reported increased level of plasma vitamin C in the experimental group as well as in control group after supplementation of tomato juice enriched with n-3 polyunsaturated fatty acid and plain tomato juice respectively<sup>[26]</sup>.

**Table 4.** “Biochemical parameters of the subjects of different groups before and after supplementation.”

Biochemical parameters	Group I		Group II		Group III	
	B	A	B	A	B	A
Vitamin C (mg %)	1.10 ± 0.16	1.30* ± 0.13	0.96 ± 0.05	2.04* ± 0.18	1.08 ± 0.11	1.58* ± 0.10
Vitamin A (µg %)	29.45 ± 2.64	28.82 ± 2.98	22.56 ± 2.18	33.81* ± 2.55	28.49 ± 3.01	32.63* ± 2.30
Vitamin E (mg %)	1.25 ± 0.13	1.33 ± 0.09	1.64 ± 0.11	1.69* ± 0.09	1.23 ± 0.10	1.38* ± 0.08
RBC-GSH (mg /g of Hb)	0.74 ± 0.04	1.08* ± 0.08	0.72 ± 0.03	1.51* ± 0.10	0.68 ± 0.02	1.29* ± 0.08
TAC (µg of TE/g)	1199.62 ± 35.20	1339.22* ± 59.20	1259.97 ± 52.40	1307.53* ± 57.80	1358.34 ± 45.30	1440.73* ± 60.90
TAC (µg of AAE/g)	228.91 ± 10.10	318.40 ± 8.80	303.98 ± 13.30	316.01 ± 12.20	323.56 ± 8.30	360.32* ± 15.20

Values are the mean ± SEM.

\* indicates significant difference at  $p \leq 0.05$ .

B: Before tomato supplementation

A: After tomato supplementation

RBC-GSH: RBC glutathione

TE: Trolox equivalent

AAE: Ascorbic acid equivalent

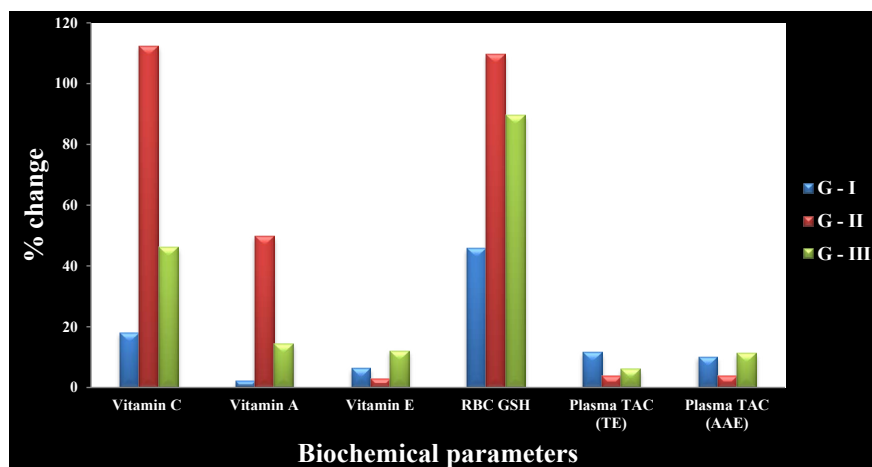
Vitamin A levels in plasma were found between 22.56 to 29.45 µg% before supplementation period (**Table 3**). The analyzed value of plasma vitamin A in group I, II and III before the supplementation period was 29.45 µg%, 22.56 µg% and 28.49 µg% respectively. After the supplementation of raw tomatoes, Group II showed 49.86 % increment in plasma vitamin A whereas subjects in Group III showed only 14.53 % increment, fed with processed tomatoes. Marja-leena et al., (2007) have also reported the increased level of plasma vitamin A in response to high tomato diet in healthy subjects<sup>[27]</sup>.

Vitamin E level in plasma was significantly higher in Group II (1.64 mg %) before the supplementations whereas it was found 1.23 and 1.25 mg% among the subjects of group I and III respectively. After the tomato supplementation vitamin E showed increasing trend in all the groups. Group I showed 6.4 % of increment in the value of vitamin E whereas group II and III showed 3 % and 12.2 % increment respectively. Seybold et al., (2004) reported that the vitamin E content was increased in thermally processed tomatoes<sup>[28]</sup>.

Glutathione (GSH) is an important non protein thiol, in conjunction with enzymes like glutathione peroxidases and glutathione-S-transferase, plays an important role in preventing the cell from toxic peroxides and aldehydes. It indirectly maintains vitamin C and E in their reduced and functional forms. The mean values of RBC-GSH and plasma GSH are presented in **Table 4**. It was found almost equal in all the groups before the supplementation and there was no any significant change among these three groups. But upon the supplementation of raw and processed tomato supplementation, it was increased significantly in all the groups. The increased level of RBC-GSH in group I has no any relation with tomato supplementation and this is may be due to their routine diet during experimental period. Group II showed highest 109.72% as compared with group I (45.64%) and group III (89.70%). Velmanguru et al., (2004) reported significant increased in liver and stomach GSH upon feeding tomatoes at the level of 0.5, 1 and 2 g / kg of body weight but the mechanism for increased RBC-GSH upon the response of tomato supplementation was not explained<sup>[29]</sup>.

The mean values of plasma TAC are presented in **Table 4**, which shows small variation among all the groups. Before the tomato supplementation TAC was ranged in between 1199.62 to 1358.34 and 228.91 to 323.56 µg equivalent to trolox and ascorbic acid / dl respectively. But after 30 days of tomato supplementation plasma TAC in all the groups were increased. Group I showed 11.62% and 10.20% increased in plasma TAC equivalent to trolox and ascorbic acid respectively. This increment was not associated with supplementation but due to their regular diet. The subjects of group II, who were fed with raw tomatoes showed 3.77% and 3.95% increment in TAC equivalent to trolox and ascorbic acid respectively. But the subjects of group III showed 6.06% and 11.36% increment in TAC equivalent to trolox and ascorbic acid respectively that were supplemented with processed tomatoes. Dietary fat has been shown to promote absorption of fat soluble antioxidants such as lycopene, principally via stimulating bile production for the formation of bile acid micelles. Ines J.P et al., (2012) suggested that the type and quantity of lipids present during digestion influence bioaccessibility of antioxidants from raw tomato<sup>[30]</sup>. Lee et al., (2000) reported no change in plasma TAC upon tomato supplementation with sunflower oil but they have reported the significant increased plasma TAC upon

tomato supplementation with olive oil<sup>[31]</sup>. In this study, tomato supplementation was carried out along with 5 gm of butter. The process of cooking which releases lycopene from the matrix into the lipid phase of the meal, increases its bioavailability, and tomato paste and tomato puree are more bioavailable sources of lycopene than raw tomatoes and increases TAC<sup>[32,33]</sup>. The percent change in all the biochemical parameters are presented in **Figure 1**.



**Figure 1.** "Percent change in the biochemical parameters of subjects in different groups."

## CONCLUSION

Tomato is a versatile fruit which is consumed all around the world wide. About 80 % of the tomatoes are being processed. Nutritionally, tomatoes are an excellent source of antioxidants, vitamin C, vitamin E, pro-vitamin A, phenolic acids and flavonoids. Along with this tomato contains a potent antioxidant, lycopene which additionally improves the total antioxidant capacity of tomatoes with other antioxidants. This study was performed with the main aim to study the effect of raw and processed tomato supplementation on plasma total antioxidant capacity. Results of the study, reveals that, the supplementation of raw and processed tomato increased plasma vitamin C and RBC glutathione significantly. Raw tomato supplementation increased plasma vitamin A whereas the processed tomato supplementation showed significant increased in plasma vitamin E. Tomato supplementation in raw as well as in the processed form did not show any significant change in plasma TAC. From the results, it can be concluded that the daily supplementation of raw or processed tomato helps in maintaining the normal plasma antioxidant status and there by helps in preventing the chronic diseases.

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