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# Effect of Purified Lycopene on Lipid Profile, Antioxidant Enzyme and Blood Glucose In Hyperlipidemic Rabbits

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## ABSTRACT

While tomato products supplementation, containing various carotenoids, including lycopene has hypolipidemic and antioxidant effect, the role of purified lycopene for the same remains unclear. Thus we tested the effect of pure lycopene powder for its effect on lipid profile, blood glucose and antioxidant enzyme in hyperlipidemic rabbits.Male New- Zealand White rabbits were used. Blood samples from all the 12 rabbits were taken for the baseline level of lipids, [Serum Total Cholesterol (TC), Low Density Lipoproteins (LDL), Serum Triglycerides (TG), High Density Lipoproteins (HDL)] blood glucose and blood superoxide dismutase(SOD). Same tests were performed in high fat diet fed (control group) and high fat diet + lycopene (10 mg/kg) (test group) after 6 weeks. There was a significant decrease in the level of serum TC, LDL – C and serum TG and an increase in serum HDL – C and antioxidant SOD after addition of lycopene to high fat diet. There was however no change in blood glucose level. Purified lycopene showed significant hypolipidemic and antioxidant activity. However, it did not show significant effect on blood glucose level.

**Key Words**

Lycopene, Hypolipidemia, Antioxidant Superoxide Dismutase, High fat diet.

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## INTRODUCTION

Despite changes in lifestyle and the use of new pharmacological approaches to lower cholesterol and blood sugar levels, cardiovascular disease (CVD) continues to be the major cause of death. Nearly million deaths are attributed to this disease.[1]Due to increasing obesity and altered dietary habits in both western and developing countries, the prevalence of cardiovascular disease due to atherosclerosis and diabetes is growing at an exponential rate.[2] It is estimated that 71.3 million Americans have at least one form of CVD.[3] Cardiovascular disease is a collective term for all the diseases of the heart and blood vessels.

There are many risk factors associated with the increased risk for cardiovascular disease (American Heart Association, 2003). High blood cholesterol levels, high blood pressure and lack of physical activity are some risk factors that can be modified (American Heart Association 2003).

Nutrition is perhaps the most significant environmental factor that has been implicated in either the development or prevention of chronic degenerative diseases.[4] The risk of heart disease is reduced significantly in populations consuming diets rich in fruits and vegetables.[5, 6, 7]

Carotenoids are fat soluble natural pigments which are synthesized by plants and are responsible for the bright colors of various fruits & vegetables. Carotenoids may protect LDL from oxidation, a process implicated in the development of atherosclerosis. The role of dietary antioxidants including vit. C, vit. E and β- carotenes in disease prevention has received much attention in recent years [8] & much work has been done on them. Recently, there is a sudden shift of attention from these antioxidants to lycopene, a carotenoid which lacks provitamin A activity due to the absence of β – ionone ring. It is present in many fruits and vegetables including watermelon, guava, apricots and papaya, but tomatoes and tomato products constitute the major source of lycopene in developed and developing countries in varying different forms. Dietary intake of tomatoes and tomato products containing lycopene has been shown to be associated with a decreased risk of chronic diseases such as cardiovascular diseases and cancer. [9, 10]

Lycopene is an antioxidant known to provide protection against cellular damage caused by reactive oxygen species. Although the antioxidant properties of lycopene are thought to be primarily responsible for its beneficial properties, evidences are accumulating to suggest that other mechanisms such as modulation of intercellular gap junction communication, hormonal & immune systems and metabolic pathway may also be involved.[11]

Although the antioxidant properties of tomato & processed tomato products have been extensively studied for the prevention of cardiovascular diseases, the beneficial effects of pure lycopene supplement is still debatable. Despite the spotlight on lycopene, it may not be protective on its own. It may only be a marker for other active substances in tomatoes or it may work with other phytonutrients to confer health benefits. So in the present study we aimed to evaluate the hypolipidemic, hypoglycemic and antioxidant effect of pure lycopene powder in hyperlipidemic rabbits.

## MATERIALS AND METHODS

Male New Zealand White rabbits (n = 12) and weighing 1.8 – 2.5 kg were used for the study. Rabbits were housed individually in standard stainless steel cages at $24^0$C with a 12 hr light: dark cycle. Rabbits were allowed free access to food and tap water. The studies were carried out in accordance with the guidelines given by Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA), New Delhi (India).The study was approved by Institutional Animal Ethics Committee (IAEC) of Padmashree Dr. D.Y.Patil Medical College, Pimpri, Pune. The rabbits were allowed to acclimatize for 1 week after which blood samples were collected from all rabbits and they were then divided into 2 different groups (n= 6) and fed one of the following diets for 6 weeks: normal rabbit chow with High fat diet [11] or normal rabbit chow with high fat diet & lycopene.

**1. High fat diet:** Constituted a mixture of coconut oil (from Marico Industries, Mumbai) and Vanaspathi ghee (from Hindustan Lever Ltd, Mumbai) procured from market.

**2. Lycopene:** Powder was purchased from Zenith Nutrition (Bangalore).The Quality analysis of lycopene was done from Bio – gen Extracts Pvt. Ltd. Bangalore.

**Method of preparation of high fat diet:**

Edible coconut oil and vanaspathi ghee mixed together in the ratio of 2:3 respectively v/v as per method of Shyamala MP et al.[11]

**Method of inducing hyperlipidemia:**

A high fat diet, consisting of coconut oil and vanaspathi ghee, in a ratio of 2:3 v/v at a dose of 5 ml/kg body weight was fed to all the animals orally, in addition to normal rabbit chow for 6 weeks.

Lycopene was given orally to one group of 6 rabbits with a feeding tube in the dose of 10 mg/kg in addition to the above diet for 6 weeks.

Fasting blood glucose was estimated by Optium Xceed glucometer. The level of serum total cholesterol, total triglyceride, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol was estimated to study the hypolipidemic activity of lycopene. In addition, plasma level of superoxide dismutase was done to study the antioxidant effect of lycopene. All the above analyses were done before and after 6 weeks in both the groups.

**Measurement of Blood glucose levels:**

Blood glucose level was estimated by putting drop of blood on test strip which was inserted into the glucometer.

**Principle:**

The Glucometer blood glucose test is based on measurement of electrical potential caused by the reaction of glucose with the reagents on the electrode of the strip. The blood sample is drawn into the tip of the test strip through capillary action. Glucose in the sample reacts with glucose oxidase and potassium ferricyanide. Electrons are generated producing a current which is proportional to the glucose in the sample. After a specific reaction time for glucometer, the glucose concentration in the sample is displayed.

**Lipid Profile:**

Lipid profile (Total cholesterol, triglyceride and HDL) was estimated manually on spectrophotometer. All the reagents for lipid profile analysis were purchased from Erba Manheim

**Measurement of total cholesterol:**

Plasma total cholesterol concentration was measured using the CHOD/PAP method (Erba Cholesterol DES assay kit) which depends on the oxidation of cholesterol by cholesterol oxidase to 7 – hydroxyl – cholesterol. The hydrogen peroxide liberated then reacts with phenol and 4 – amino antipyrine in the presence of peroxidase to yield a quinoneimine chromophore that is measured at 505 nm.

**Measurement of triglyceride:**

Plasma total triglyceride concentration was measured using the GPO – Trinder method (Erba Triglyceride DES assay kit) in which triglycerides undergo hydrolysis in the presence of lipoprotein lipase to form glycerol & Free Fatty Acids. Glycerol reacts with ATP to form glycerol – 3 – phosphate which is oxidized to dihydroxyacetone phosphate. The hydrogen peroxide liberated reacts with 4 – amino antipyrine and 3, 5 – dichloro – hydroxybenzene sulfonate to yield a quinoneimine chromophore that is measured at 505 nm.

**Measurement of HDL:**

Plasma HDL cholesterol concentration (using Erba Liquixx HDL – Cholesterol kit) was defined as that fraction of total cholesterol that remained in solution after precipitation of LDL and VLDL cholesterol with magnesium chloride/Phosphotungstic acid reagent added to the plasma sample. The plasma sample was centrifuged and the clear supernate assayed by the method used for plasma total cholesterol concentration.

**Measurement of LDL:**

Low density lipoprotein was estimated according to the Friedewald's equation. [12]

LDL = Total Cholesterol – TG/5 - HDL.

**Measurement of superoxide dismutase:**

Measurement of superoxide dismutase was done by using the method of Marklund et al.[13]

| Reagent | Control | Test |
|---|---|---|
| Tris buffer | 3.0 ml | 2.95 ml |
| Pyrogallol | 0.3 ml | 0.3 ml |
| Serum | ------- | 0.05 ml |

Mix and measure the absorbance continuously for 4 min at 420 nm at 30 sec. interval.

**Calculation:**

Absorbance reading to be taken for calculation is the reading at 3.5 mi minus reading at 0.5 min. If absorbance reading of control is A and test is B then:

$$SOD = \frac{A - B}{A \times 50} \times \frac{100}{0.05} \text{ in units / ml.}$$

**Statistical analysis**

The statistical analysis of the study was done using statistical package SAS. (Version 9.2 for Windows). All the results were expressed as Mean ± SEM. Student's'‘t’ test(paired ‘t’ test) was used to assess the statistical significance of the results between control group (baseline) and high fat diet group to see the effect of high fat diet on lipid profile. Statistical analysis of the data between the high fat diet group and High fat diet + lycopene group was done by using one – way analysis of variance (ANOVA). The probability level less than 0.05 (< 0.05) was considered statistically significant.

## RESULTS AND DISCUSSION

There was no significant difference in the mean body weight of both the groups. Baseline level of blood glucose, lipid profile & superoxide dismutase were also comparable in both the groups. After the baseline sample was taken, rabbits were divided into 2 groups:

1. High fat diet group
2. High fat diet & lycopene group

Table 1 shows the effect of high fat diet on the lipid profile, fasting blood glucose & superoxide dismutase which is taken as a parameter for its antioxidant activity. The high fat diet resulted in a significant increase in blood lipid levels (TC, LDL – C & TG). There was also a significant decrease in the level of HDL – C and superoxide dismutase. No significant difference was found in fasting blood glucose after high fat diet.

**Table 1**: **Comparison between baseline & High fat diet group at 6 weeks.**

| Parameter | Mean ± SEM Baseline | Mean ± SEM High Fat Diet | P - value |
|---|---|---|---|
| BSL (mg/dl) | 100.5 ± 1.76 | 103.5 ± 1.56 | 0.232 |
| TC (mg/dl) | 106.6 ± 2.06 | 219.9 ± 7.38 | < 0.0001* |
| HDL (mg/dl) | 36.73 ± 1.76 | 25.01 ± 1.17 | < 0.0001* |
| TG (mg/dl) | 93.97 ± 1.41 | 149.5 ± 4.11 | < 0.0001* |
| LDL (mg/dl) | 51.09 ± 0.39 | 164.9 ± 5.47 | < 0.0001* |
| SOD (units) | 3.233 ± 0.054 | 2.292 ± 0.050 | < 0.0001* |

* indicates significant findings

BSL (Blood Sugar Level), TC (Total Cholesterol), HDL (High Density Lipoprotein), TG (Triglyceride), LDL (Low Density Lipoprotein), SOD (Super Oxide Dismutase

Serum lipid levels were significantly less in group 2, after additional supplementation with lycopene as compared to high fat diet (HFD) group. We observed significant less level of serum TC, LDL – C & TG while there was a significant increase in HDL – C and serum superoxide dismutase levels with addition of lycopene. Table 2 shows the effect of additional supplementation of lycopene in high fat diet group.

**Table 2: Comparison between HFD & HFD + lycopene groups at 6 weeks.**

| Parameter | Mean ± SEM (High Fat Diet) | Mean ± SEM (HFD+Lycopene) | P - value |
|---|---|---|---|
| BSL (mg/dl) | 103.5 ± 1.56 | 100.5 ± 1.61 | 0.116 |
| TC (mg/dl) | 219.9 ± 7.38 | 161.4 ± 2.10 | < 0.0001* |
| HDL (mg/dl) | 25.01 ± 1.17 | 29.00 ± 1.13 | 0.007* |
| TG (mg/dl) | 149.5 ± 4.11 | 123.6 ± 2.87 | < 0.0001* |
| LDL (mg/dl) | 164.9 ± 5.47 | 106.9 ± 0.77 | < 0.0001* |
| SOD (units) | 2.292 ± 0.05 | 2.987 ± 0.03 | < 0.0001* |

* indicates significant findings

Tomato is one of the most widely consumed fruits/vegetables all over the world. It has been estimated that in America the average annual consumption of fresh tomatoes is approximately 8 kg per person and of processed tomato products is 31 kg per person. This makes tomato the most frequently consumed canned vegetable and therefore considered the topmost dietary source of

lycopene. As a powerful antioxidant, lycopene helps neutralize harmful free radicals which are implicated in cancer, heart disease, macular degeneration and other age related illnesses. In a latest Harvard study of more than 28000 women, those with the highest blood lycopene level were about half as likely to develop heart disease over 5 years as women with the lowest levels. Research also suggests that lycopene may aid blood pressure and bone health.

Whether lycopene supplements are as beneficial as whole food sources is debatable. In our study, we found beneficial effects of pure lycopene powder in hyperlipidemic rabbits. There was a significant decrease in serum TC, LDL – C & total TG and a significant increase in serum HDL – C Figure 1. This suggests that lycopene may play a role in the improvement of serum lipid profile and may possibly enhance LDL – C degradation. What are the mechanisms behind the cholesterol lowering effects of lycopene? Increased fecal cholesterol excretion, together with reduced liver 3- Hydroxy – 3 – methyl glutarine coenzyme A (HMG CoA) reductase activity was shown after dietary lycopene intake in rabbits suggesting decreased intestinal cholesterol absorption and biosynthesis .[1] Reasons for the effect of lycopene on plasma lipid levels in human intervention studies are not consistent. Supplementation with tomato extract capsules (4 mg lycopene) daily for 6 months decreased total cholesterol and LDL cholesterol levels in post menopausal women.[14] No effects on blood lipid levels were obtained after supplementation with a tomato extract containing 15 mg lycopene daily for 8 weeks in mild hypertensive patients.[15] A recent meta- analysis of human intervention trials revealed significant reduction in total and LDL cholesterol only at doses of more than or equal to 25 mg of lycopene daily. Doses of less than 25 mg lycopene had no effect on serum cholesterol levels. HDL cholesterol was not changed by lycopene intake independently of dosage given.[16] Reactive oxygen species (ROS) and the related oxidative damage have been implicated in the pathogenesis of various human chronic diseases.[17, 18, 19, 20] Lycopene is one of the most potent antioxidants[21] and has been suggested to prevent carcinogenesis and atherogenesis by protecting critical biomolecules including lipids, LDL cholesterol, proteins & DNA.[22, 23, 24] Several studies have indicated that lycopene is an effective antioxidant and free radical scavenger. Lycopene because of its high number of conjugated double bonds, exhibits higher singlet oxygen quenching ability compared to beta carotene or alpha tocopherol.[25] Oxidative modification of LDL is hypothesized to be the key step in the atherogenetic process, and LDL associated antioxidants provide protection against this oxidation. In vitro, lycopene and other carotenoids are able inhibit oxidation of LDL.[26]

In our study, as shown in Table 3 Figure 2 there was a significant increase in level of superoxide dismutase as a marker of its antioxidant effect. This finding is in common with the previous

study.[27] SOD is arguably body's most crucial antioxidant as it is responsible for disarming the most dangerous free radicals of all the highly reactive superoxide. It reduces the radical superoxide ($O_2^-$) to form hydrogen peroxide and oxygen ($H_2O_2$ & $O_2^-$). Although $H_2O_2$ is also a prooxidant compound, it is subsequently converted by enzyme catalase and glutathione peroxidase to simple water and oxygen. So, by strengthening the body's primary antioxidant system, this novel SOD boosting supplement may offer the powerful free radical protection and may play a protective role in reducing the oxidative stress implicated in atherosclerosis and other life threatening diseases. There was however no significant difference in fasting blood sugar level in lycopene and high fat diet (HFD) group (Table 2 Figure 1). This suggests that though lycopene has hypolipidemic and antioxidant effects but it lacked the hypoglycemic activity.

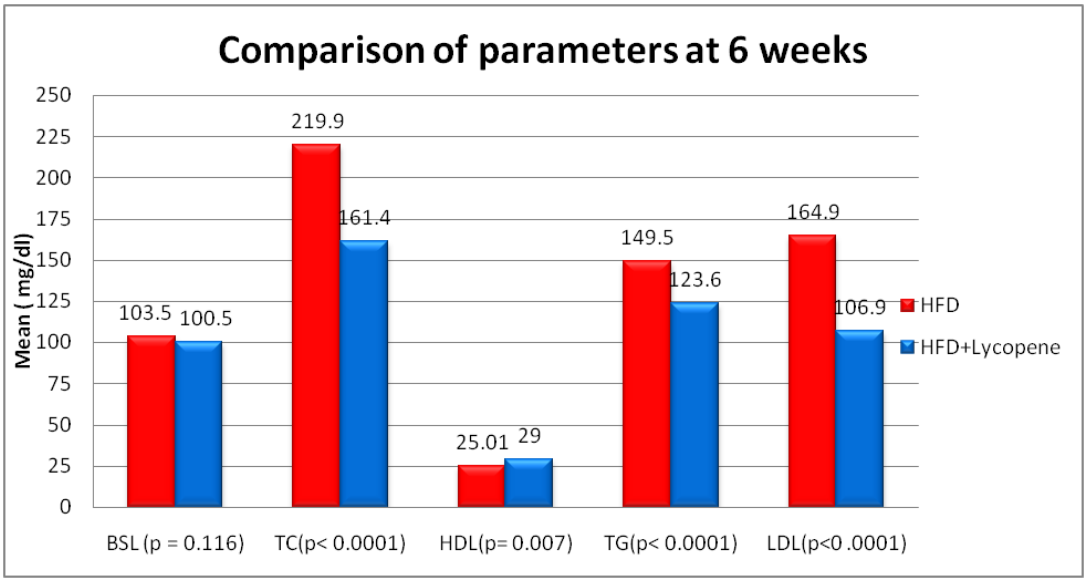


**Figure 1: Comparison of parameters at 6 weeks.**

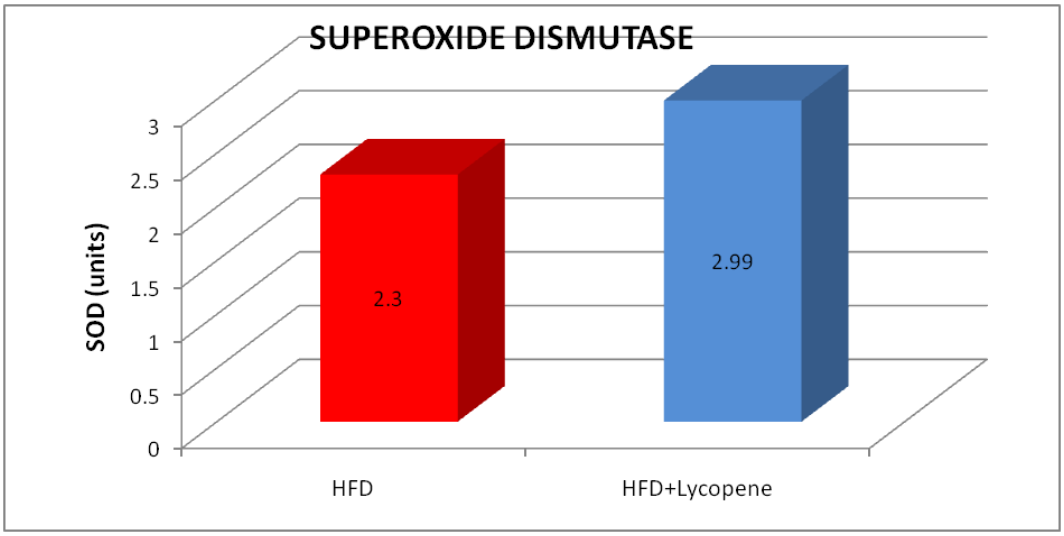


**Figure 2: Effect on level of Superoxide Dismutase at 6 weeks.**

**Table 3: Effect of study drugs on Blood Antioxidant (Superoxide Dismutase).**

| Group | Total Superoxide (Units) |
|---|---|
| High Fat Diet (5 ml/kg) | 2.292 ± 0.05 |
| High Fat Diet (5 mg/kg) + Lycopene (10 mg/kg) | 2.987 ± 0.03 |
| P value < 0.0001 which is significant. | |

## CONCLUSION:

These findings suggest that lycopene may have considerable therapeutic benefit as an antioxidant and hypolipidemic agent but may not be used as a hypoglycemic agent.

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