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**Original Article**

# *Effect of Lycopene Administration on the Antioxidant Status of Hypercholesterolemic Wistar Rats (Rattus Norvegicus)*

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***ABSTRACT***

*An imbalance between oxidants and antioxidants causes oxidative stress. Antioxidants such as vitamins C and E, known to prevent various diseases, can help reduce oxidative stress. The antioxidant lycopene in fruits can prevent carcinogenesis and atherogenesis. The objective of this study is to assess the impact of administering lycopene at doses of 0.36, 0.72, and 1.08 mg/day from tomato (Lycopersicum esculentum) fruit extract on the antioxidant levels (Vitamin C, E, and GPx) in hypercholesterolemic Wistar rats (Rattus norvegicus). The research methods of this study employ true experiment designs, LSD post hoc, control, and treatment groups. Experimentally, 28 rats with the Wistar strain were assigned to control and treatment groups. Hypercholesterolemic rats were fed high-fat, high-cholesterol with 0.35 ml/day cholesterol crystals. Vitamin C, vitamin E, and GPx levels were measured. The result of this study shows lycopene increased both vitamin C and E ($P_3 > P_2 > P_1 > P_0$). A post hoc LSDV statistical test of vitamin C, vitamin E, and GPX levels shows a significant difference ($p = 0.00$). The conclusion of this study found that administering a dosage of 0.36 mg/head/day of lycopene to those with high cholesterol levels benefits the body's antioxidant status, thereby improving the overall ability to counteract oxidative stress.*

**Keywords:** *Lycopene, Free Radicals, Antioxidant.*

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## INTRODUCTION

The National Household Health Survey reveals that high LDL cholesterol levels are atherogenic, while high HDL cholesterol levels protect against CHD. The National Household Health Survey shows that Cardiovascular Disease (CVD) is the most common degenerative disease and leading killer in industrialized countries, ranking first as the cause of death for people over 40 years old[1]. CHD, closely related to atherosclerosis, is the leading cardiovascular disease in productive age[2]. Understanding cholesterol levels prevents deadly diseases like CHD[3].

Oxidative stress, a disease trigger factor, increases the number of free radicals and Reactive Oxygen Species (ROS) essential for cell damage[4]. Enzymatic antioxidants like superoxide dismutase, catalase, and glutathione peroxidase, while non-enzymatic antioxidants like alpha-tocopherol and ascorbic acid, play a crucial role in controlling ROS[5-9]. Exogenous sources of antioxidants, such as vitamins like vitamin C and ß-carotene, are also needed to minimize oxidative stress[10]. Vitamin C, found in Lycopene, works with vitamin E to inhibit oxidation reactions, protecting important cell

biomolecules, including lipids, proteins, and DNA. These antioxidants, combined with vitamins like β-carotene and β-tocopherol, are believed to prevent carcinogenesis and atherogenesis.

Free radicals cause cell damage through peroxidation of lipid components, DNA damage, and protein modification[11-16]. This damage is linked to various diseases, including heart disease, pain, inflammation, cancer, diabetes, Alzheimer's disease, liver damage, and glaucoma[17]. Antioxidants, such as lycopene in fruits, protect important cell biomolecules and prevent carcinogenesis and atherogenesis[18]. Long-term exposure to synthetic antioxidants can cause side effects and increase the risk of carcinogenesis[19]. Consuming natural antioxidants from fruits, vegetables, and flowers can help prevent degenerative diseases[14-16,20].

The effect of lycopene, vitamin C, and vitamin E on hypercholesterolemia in rats is investigated in this study. We picked these rats for their hypercholesterolemia-causing abilities because of their omnivorous diet and treatment resistance. This research aimed to discover how lycopene affected lipid profiles, antioxidant status, and the protection of LDL cholesterol from oxidation, foam cell formation, and atherosclerotic lesions.

## METHOD

The experiment conducted at the experimental research base involved dividing rats into control and treatment groups using simple randomization[21,22]. The rats were administered a diet rich in high fat and high cholesterol and lycopene treatment to enhance their antioxidant levels. The data analysis involved using descriptive and inferential statistical tests; specifically, ANOVA and LSD tests were employed for comparison purposes. The study employed SPSS 25 for Windows for data processing and presentation[23].

The Federer formula calculates the sample size for each treatment group using the inequality (n-1) (t-1) > 15 (n = sample size, t = sample group size)[24]. According to the calculation, each treatment group had at least 6 participants. This study had four treatment groups of 7 mice each. Therefore, a total of 28 mice were sampled. The lycopene administration doses 0.36 mg/day, 0.72 mg/day, and 1.08 mg/day served as the independent variable. At the same time, the antioxidant status, vitamin C, vitamin E, and glutathione peroxidase levels were the dependent variables. Each dependent variable is associated with an independent variable that either has an influence on it or is a consequence of it[25].

The extract technique is used to administer lycopene from tomato (*Lycopersicon esculentum*) fruit extract to rats[26,27]. The dosages varied between control and treatment groups, being 0.72 mg/head/day, 1.08 mg/head/day, and 1.44 mg/head/day due to conversion from human doses. Hypercholesterolemic rats, aged 12 weeks, have total cholesterol levels above 120 mg/dl, which are measured based on serum cholesterol levels after high fat, high cholesterol feed.

Procedure research includes animal preparation experiments, dose calculation, and feed preparation.

## RESULTS

The rats' mean body weight was 180-200 grams, with 185.8 grams meeting the inclusion criteria. Normal rats' baseline cholesterol averages 111.45 mg/dl. The high-fat, high-cholesterol diet raised blood serum cholesterol to the inclusion criteria (>120 mg/dl) with a mean value of 221.8 mg/dl before lycopene. The mean cholesterol dropped to 151.9 mg/dl after lycopene administration. Twenty-eight rats consumed isocaloric food before and after lycopene administration.

Plasma lycopene levels increased in most treatment groups for mg/head/day (P1 = 0.72, P2 = 1.08, P3 = 1.44) from 54 UI/L to 67, 76, and 97, except in group P0 (control group), which decreased from 54 to 53. P3 had almost twice the plasma lycopene increase of the control. Plasma lycopene levels rise with dose.

The study results on antioxidant status in the form of measurement results of vitamin C, vitamin E, and glutathione peroxidase levels measured enzymatically are described and included in Table 1.

**Table 1. Mean and Standard Deviation of Vitamin C, E, And GPx Levels**

| Variable | Control ($P_0$) | | Treatment 1 ($P_1$) | | Treatment 2 ($P_2$) | | Treatment 3 ($P_3$) | | $\Sigma$ | | *p* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Mean** | **Std** | **Mean** | **Std** | **Mean** | **Std** | **Mean** | **Std** | **Mean** | **Std** | |
| Vit. C Content | 0,5 | 0,09 | 0,81 | 0,08 | 1,16 | 0,69 | 1,46 | 0,73 | 0,99 | 0,38 | 0,00** |
| Vit. E Level | 7,45 | 0,49 | 12,45 | 0,33 | 16,16 | 0,33 | 19,41 | 0,33 | 13,87 | 4,543 | 0,00** |
| GPx Level | 93,38 | 1,53 | 80,77 | 1,83 | 54,93 | 1,52 | 31,74 | 1,53 | 65,36 | 24,45 | 0,00** |

Differences between the two treatment groups on vitamin C, vitamin E, and GPx levels can be seen in Table 2.

**Table 2. LSD Post Holc Test in Vitamin C, E, And GPx Levels Between Two Treatment Groups**

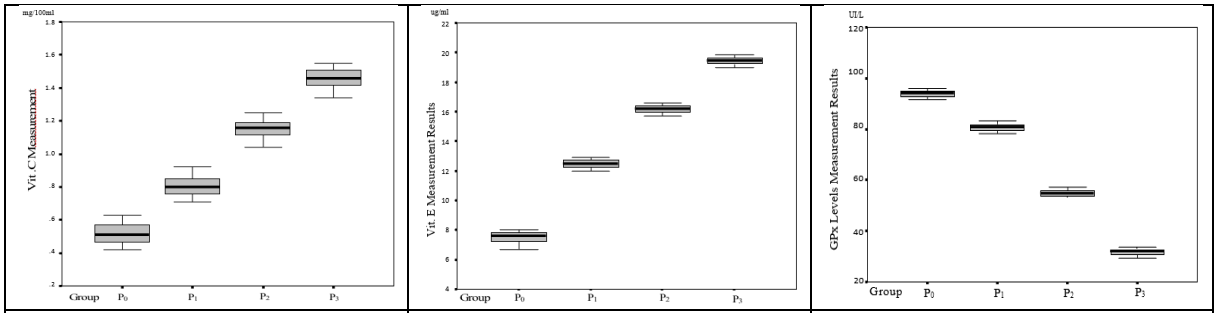
| | Treatment Groups | | | | | |
|---|---|---|---|---|---|---|
| **Variable** | **K-$P_1$** | **$P_1$-$P_2$** | **$P_2$-$P_3$** | **K-$P_2$** | **K-$P_3$** | **$P_1$-$P_3$** |
| Vit. C Content *(p)* | 0,00** | 0,00** | 0,00** | 0,00** | 0,00** | 0,00** |
| Vit. E Level *(p)* | 0,00** | 0,00** | 0,00** | 0,00** | 0,00** | 0,00** |
| GPx Level *(p)* | 0,00** | 0,00** | 0,00** | 0,00** | 0,00** | 0,00** |

*** LSD is highly significant.*

Data from Table 2 shows that the mean vitamin C levels in the treatment groups ($P_1$, $P_2$, and $P_3$) were higher than the mean vitamin C levels in the control group ($P_0$). Anova test results showed a significant difference between the four treatment groups with p=0.00. LSDV post hoc statistical test indicated that there was also a substantial difference between the two groups in all treatments with p=0.00.

The mean vitamin E levels in the treatment groups ($P_1$, $P_2$, and $P_3$) were higher than the mean vitamin C levels in the control group ($P_0$). The ANOVA test results show a significant difference between the four treatment groups with p=0.00. LSD post hoc statistical test indicated that there was also a substantial difference between the two groups in all treatments with p=0.00.

The data shows that the mean levels of glutathione peroxidase were the opposite; in the control group (P0), the mean levels of glutathione peroxidase were higher than the mean levels of glutathione peroxidase in the treatment groups (P1, P2, and $P_3$). The ANOVA test results showed a significant difference between the four treatment groups with p=0.00. LSD post hoc statistical test indicated that there was also a substantial difference between the two groups in all treatments with p=0.00.




(a) (b) (c)

***Figure 1.*** *(**a**) Boxplot of vitamin C measurement results; (b) Boxplot of vitamin E measurement results; (**c**) Boxplot of the GPx levels measurement results of GPx levels.*

The boxplot in Figure 1. (a) shows that the median vitamin C in the treatment group ($P_1$, $P_2$, and $P_3$) is higher than the median vitamin C in the control group ($P_0$). Figure 1. (b) shows that the median vitamin E in the treatment group (P1, P2, and $P_3$) is higher than the median in the

control group ($P_0$). Figure 1. (c) shows that the median glutathione peroxidase decreased, with the control group (P0) having the highest median glutathione peroxidase compared to the treatment group.

## DISCUSSION

In this study, male rats (*Rattus Norvegicus* Wistar strain) were grouped into four treatment groups; each group consisted of 7 rats, so the total sample was 28. With an average rat body weight of 180-200 grams at 12 weeks and cholesterol levels < 120 mg/dl. Experimental animals were kept in individual cages and cleaned every day. The cage temperature was 28-32$^{o}$C, and there was adequate air circulation and light.

This study used 1% crystalline cholesterol in feed as much as 18 gr/head/day for 20 days in rats to change the cholesterol levels of rats from 85.8 mg/dl + 12.9 to 112.2 mg/dl + 21.2 [28]. Feeding high-fat, high cholesterol in the form of 10% lard and pure cholesterol as much as 10 gr/kg in this study was shown to increase cholesterol levels within 14 days. Furthermore, the treatment will be given lycopene doses of 0.36 mg/day, 0.72 mg/day, and 1.08 mg/day in each group so that changes in antioxidant status can be known, which include levels of vitamin C, vitamin E, and glutathione peroxidase (GPx).

Research shows the mechanism of lycopene in protecting native LDL from oxidation and suppressing cholesterol synthesis[29]. If its protective ability is reduced, native LDL will develop into LDL-ox, which has the characteristics of containing lipid peroxides and other degradation products, apoprotein B, which is degraded through scavenger receptors, causing lipid accumulation in macrophages, low levels of fat-soluble antioxidant vitamins and has immunogenic and biologically active properties[30].

According to epidemiological studies, consuming plenty of tomatoes (Lycopersicon esculentum) and their processed derivatives can increase plasma levels of lycopene[31]. Lycopene levels decreased in the control group, suggesting that lycopene does not raise plasma levels. A diet rich in fat and cholesterol continuously causes oxidative damage so that the antioxidant effect of lycopene may be reduced. Different doses of lycopene increased plasma levels, suggesting an increase in antioxidant ability. Consumption cannot determine circulatory lycopene levels. Bloodstream plasma and adipose tissue lycopene levels are better indicators of disease prevention than dietary consumption.

The levels of various doses of lycopene on vitamin C, E, and GPx in this study show that lycopene interacts directly with other antioxidants through oxidative mechanisms. The more lycopene is given, the higher the levels of vitamin C and E compared to the control ($P_3>P_2>P_1>P_0$) seen in the results of this study. GPx levels have been reduced, with the lowest being 0.36 mg/day ($P_1$).

Vitamin C is a water-soluble vitamin that can only eliminate free radicals in liquid media. Vitamin C can suppress free radicals that will attack lipids[32]. This vitamin reacts with superoxide, hydroxyl anion, and lipid hydroperoxides as a free radical scavenger. Vitamin C, a chain-breaking antioxidant, regenerates reduced vitamin E. Vitamin C acts as a secondary antioxidant by maintaining reduced glutathione. Chain reactions in lipid peroxidation can be stopped by vitamin E by giving a single electron to two consecutive responses to form a stable oxidized compound[33]. Giving lycopene in this study is expected to increase vitamin E levels so that its ability as an antioxidant is even greater. If lycopene, vitamin C, and vitamin E levels rise, the decrease in GPx levels may be positive (feedback mechanism). Excess lycopene can become pro-oxidants in an antioxidant balance mechanism, so GPx is needed to bind them[34].

## CONCLUSION

The study results indicate that vitamins C and E, especially at 0.36 mg, possess antioxidant characteristics, which help maintain and improve the body's antioxidant capacity. Vitamin C is a secondary antioxidant that helps maintain lowered glutathione levels. In contrast, a higher intake of lycopene leads to increased levels of vitamin E, indicating a mutually beneficial connection between antioxidants. Although there was a drop in GPx levels due to the dosage effects, this reduction could be helpful if there is a significant improvement in other antioxidant statuses.

This improvement may reduce the reliance on GPx to counteract pro-oxidants. In hypercholesterolemic situations, injecting 0.36 mg/head/day of lycopene favors antioxidant

status, improving the body's ability to counteract oxidative stress. The study emphasizes the complex interaction between different antioxidants and their ability to work together to enhance the body's antioxidant defense mechanisms. This is especially important in situations of oxidative stress, such as hypercholesterolemia.

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