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**Title:** **Cardioprotective Effect of *Allium sativum* on C-Reactive Protein and Cardiac Structure in Rabbits**

**Author (s):** Iza Tahreem[1], Irum Naureen[1], Aisha Saleem[1], and Muhammad Naeem[2]

**Affiliation (s):** [1]Minhaj University Lahore, Pakistan
[2]Institute of Research and Information, Mirpur, Pakistan

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# Cardioprotective Effect of *Allium sativum* on C-Reactive Protein and Cardiac Structure in Rabbits

Iza Tahreem[1], Irum Naureen[1*], Aisha Saleem[1], and Muhammad Naeem[2]

[1]School of Zoology, Minhaj University Lahore, Pakistan
[2]Institute of Research and Information, Mirpur, Pakistan

**Abstract**

**Background.** Diet and lifestyle comprise major risk factors in the development of various human diseases, including atherosclerosis. C- Reactive protein (CRP) is one of the possible markers of vascular inflammation. High CRP levels can predict the long-term risk of cardiovascular diseases. The current study aimed to evaluate the ameliorating effect of *Allium sativum* against high-fat diet-induced damage to heart structure and CRP levels.

**Methodology.** A total of 16 rabbits were randomly assigned to 4 groups (n=4 each). Group 1 served as the control group and it was fed with standard diet. Whereas, the remaining 3 groups were experimental groups. Group 2 was fed with high fat diet, Group 3 was fed with high fat diet supplemented with 5% *Allium sativum*, and Group 4 was fed with high fat diet supplemented with 10% *A. sativum*. The experiment was conducted for 4 weeks.

**Results.** Mild myocardial congestion was observed in the heart tissues of rabbits in Group 2 fed with high fat diets. Group 3 and Group 4, fed with 5% and 10% *A. sativum* respectively and with a high fat diet, showed no myocardial congestion. This indicates the cardio-protective potential of *A. sativum*. Group 2 showed a significant ($p$<0.05) increase in CRP levels as compared to control.

**Conclusion.** The results showed that *A. sativum* demonstrated a beneficial effect on both heart structure and CRP levels, suggesting its potential as an adjunct therapy for cardiovascular diseases.

**Keywords:** *Allium sativum,* cardiovascular diseases, C-reactive protein, heart histopathology, high fat diet, myocardial congestion

**Highlights**

- *Allium sativum* has beneficial effects on heart structure after the consumption of cholesterol rich diet.
- *Allium sativum* reduces CRP levels after the consumption of high fat diet.

## 1. INTRODUCTION

*Allium sativum* is considered a medicinal plant due to its preventive characteristics in regulating blood pressure, curing cardiovascular diseases, and lowering blood sugar and cholesterol levels. Further, it plays an effective role against fungal, viral, bacterial, and other parasitic infections. Various organosulphur compounds, such as diallyl thiosulfonate (Allicin), S-allyl-cysteine sulfoxide (Alliin), diallyl sulfide, dially disulfide,

* Corresponding Author: dr.irumnaureen@mul.edu.pk

diallyl trisulfide, ejoene, and S-allyl-cysteine are present in *A. sativum.* Due to the presence of these compounds, it plays an important pharmaceutical role in various biological activities including antibacterial, antiviral, antifungal, antiprotozoal, antioxidant, anti-inflammatory, and anticancer activities [1]. Organosulphur compounds cause the release of hydrogen disulfide from red blood cells [2]. Hydrogen disulfide is a naturally occurring cell signaling molecule. It has many cardio-protective effects including vascular smooth muscle relaxation and protection against oxidative damage. Also, it plays an important role in reducing blood pressure.

An important aspect of preventing cardiovascular diseases is regulating the plasma level of cholesterol [3]. Different pharmaceuticals are used in the treatment of cardiovascular diseases. However, due to their prolonged usage and adverse effects, herbal medicine might be considered as an appropriate alternative remedy. Various supplements of garlic have a remarkable impact on cholesterol, high density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol levels. Garlic and garlic-based preparations are beneficial in controlling plasma cholesterol levels, as well as preventing the development of atherosclerosis [4]. A high CRP level increases the risk of heart attack. Sulphur containing phytochemicals are present in garlic and they play an important role in reducing CRP levels [5].

Chronic diseases including atherosclerosis and type 2 diabetes are characterized by inflammation. A high fat diet can affect oxidative stress [6]. Both low fat diet and high fat diet have been linked to increased levels of oxidative stress indicators in rabbits and rats [7]. A high fat diet was shown to cause inflammation in both diabetic and healthy humans, as well as in animals [8]. It evoked hyperlipidemia and also caused damage to the carotid artery [9]. Inflammation is more associated with a high fat diet, while anti-oxidant vitamins inhibit inflammation. Such a diet leads to inflammation and high CRP levels [10]. The current study aimed to analyze the effects of high fat diet on CRP levels and cardiac histopathology. It also aimed to evaluate the beneficial effects of *A. sativum* on CRP levels and cardiac histopathology in rabbits after the consumption of high fat diet.

## 2. MATERIALS AND METHODS

### 2.1. Animals, treatment & experimental design

The study was conducted over a period of 4 weeks with daily supplementation of *Allium sativum. Allium sativum* (5% and 10%) indicates the weight percentage of the diet given to experimental group. Experiment was done in Animal House, Zoology Department of Minhaj University Lahore. Rabbits were kept in suitable and big wooden cages; good environmental conditions were provided to the rabbits. Cleanliness was maintained in animal house. The temperature and humidity of Animal house were continuously recorded with digital thermograph, temperature was 27±2 ºC and humidity was 40% to 50% in the animal house.

Ethical approval was taken to study from ethical approval committee of Minhaj University Lahore. Rabbits the age of 4 to 5 months were used for the experiment. 16 rabbits were randomly divided into 4 groups (n=4). The first group or Group 1 was labeled as Control Group (Go) and 3 groups were labeled as experimental groups (G1, G2 & G3). Commercially synthesized feed (Pellet no 44) was given to the rabbits and feed was rich in proteins, vitamins, essential minerals and multivitamins. Fresh

and clean water was also provided twice a day to the rabbits. Control group (Go) was given 100 g simple feed (Pellet no 44) twice a day. Experimental group (G1) fed 82g of simple diet (Pellet no 44) mixed with 8ml of beef fat oil and 10 ml of corn oil twice a day. Experimental group (G2) was given 77g simple diet (Pellet no 44) mixed with 8ml of beef fat oil,10 ml of corn oil and 5g paste of raw garlic. Experimental group (G3) fed 72 g of simple diet (pellet no 44) mixed with 8ml of beef fat oil, 10 ml of corn oil and 10 g paste of raw garlic.

Body weight of all rabbits was measured before and after the experiment. After 30 days of experiment, blood collected from the marginal ear vein (Antecubital vein) in clean tubes containing acid citrate dextrose then sample was centrifuged for 15 minutes at 3000 rpm. After centrifugation, blood sample was allowed to clot for the separation of sera. Serum was stored in eppendorf tubes for CRP test. At the end of experiment all rabbits were slaughtered and dissected. Organs were exposed and removed with the help of scissors and forceps (Figure1). Organs weights were also recorded at the end of experiment.

CRP test was done with Enzyme Linked Immunosorbent Assay (ELISA). Heart histopathology was also examined for this purpose heart was removed from body. Heart tissues were processed in automatic processor embedded in paraffin and then sectioned with the help of manual rotary microtome and stained with hemotoxyline and eosin by normal procedure for light microscopy under various magnifications.

### 2.2. Statistical analysis

The data was analyzed by using Graph Pad version 9 by applying unpaired t-test statistics and finding out the mean, standard error mean and p value for each parameter. *p* values indicated the significant difference of food consumption, CRP levels and heart weight among control and experimental groups. These differences were considered significant at $p<0.05$, $p<0.01$, $p<0.001$, $p<0.0001$.

## 3. RESULTS

### 3.1. General Observations

Rabbits of control group (Go) fed simple diet and they were active throughout the experiment while rabbits of experimental group (G1) fed high fat diet along simple diet so less food consumption was observed in them and they were also obese due to intake of fatty components. Moreover, they had hard and rough fur (Fig.2) while rabbits of other groups had soft and silky fur.

Experimental group (G3) was given simple diet along high fat diet and 5% of *Allium sativum* while experimental group (G4) fed simple diet along high fat diet and 10% of *Allium sativum*. A gradual increase in body weight was observed in them and some curing effects were also observed in them.

Feed consumption over the period of 4 weeks of experiment was also observed. It was observed that feed consumption was low in first week, in second week feed consumption was more than first week. In third week feed consumption was more than first and second week. In forth week feed consumption was high than first, second and third week (Fig.3). When heart weight was measured it was observed that rabbits of experimental group (G1) had higher heart weight as compare to the other groups due to deposition of fat. Control group (Go) had normal CRP level. As experimental group (G1) fed high fat diet along simple diet therefore their CRP level

was extremely high as compare to other groups (Go, G2, G3). Experimental group (G2) had low CRP level as compare to experimental group (G1), which suggested the curing effect of *Allium* sativum. It was observed that CRP level of experimental group (G3) was low as compared to experimental group (G1) as well as experimental group (G2) which suggested that 10% garlic had more curing effect than 5% garlic

**Table 1.** Feed Consumption Values (Mean and SEM) of Control (Go) and Experimental Groups (G1, G2, G3)

| Groups | Week 1 | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|
| Control (Go) | 193.42±2.860 | 196.428±1.797 | 194.857±1.981 | 195.857±2.186 |
| Ex G1 | 179±3.380 a** | 187.142±1.944 a** | 193.428±0.895 | 193.857±1.203 |
| Ex G2 | 169.142±3.217 a*** | 179.428±1.944 a**** b** | 183.428±2.202 a**b** | 186.428±1.250 a** b** |
| Ex G3 | 165±3.258 a****b** | 178.857±1.944 a****b** | 180±0.856 a****b**** | 178.714±1.584 a**** b**** c** |

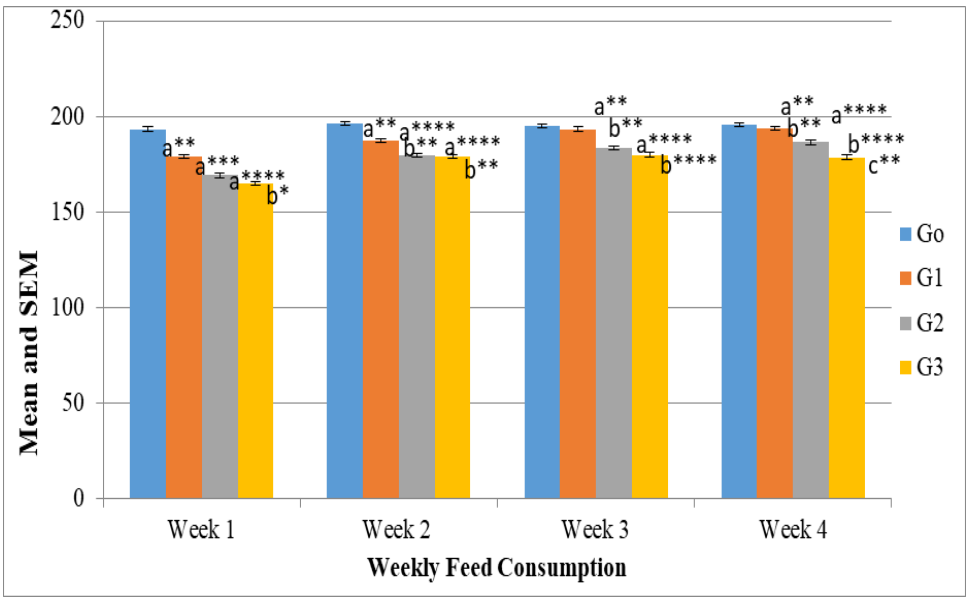


**Figure1.** Graphical Representation of Weekly Feed Consumption Between Control (Go) and Experimental Groups (G1, G2, G3).

Histogram showing the comparison of feed consumption between rabbits of control group (Go) with rabbits of experimental groups (G1, G2, G3). The Horizontal axis represents weekly feed consumption (x-axis) while vertical axis (y-axis) represents the mean and standard error mean values. Asterisks show the significant difference $p<0.05$*, $p<0.01$**, $p>0.001$***, $p>0.0001$**** between feed consumption of control group (G1) and experimental groups (G1, G2, G3) as shown in Figure 3 and Table 1.

**Table 2.** Body Weight Values, Heart Weight Values, CRP Levels (Mean and SEM) of Control (Go) and Experimental Groups (G1, G2, G3)

| Groups | Control (Go) | Ex G1 | Ex G2 | Ex G3 |
|---|---|---|---|---|
| Weight Gain | 0.268±0.051 | 0.301±0.062 | 0.082±0.039 a*b** | 0.150±0.055 |
| Heart Weight | 34.042±3.030 | 37.857±0.983 | 30.437±3.192 a** | 30.075±1.313 a**** |
| CRP level | 0.74 ± 0.111 | 1.997 ± 0.173 a*** | 1.167 ± 0.135 a*** | 0.93 ± 0.063 a** |

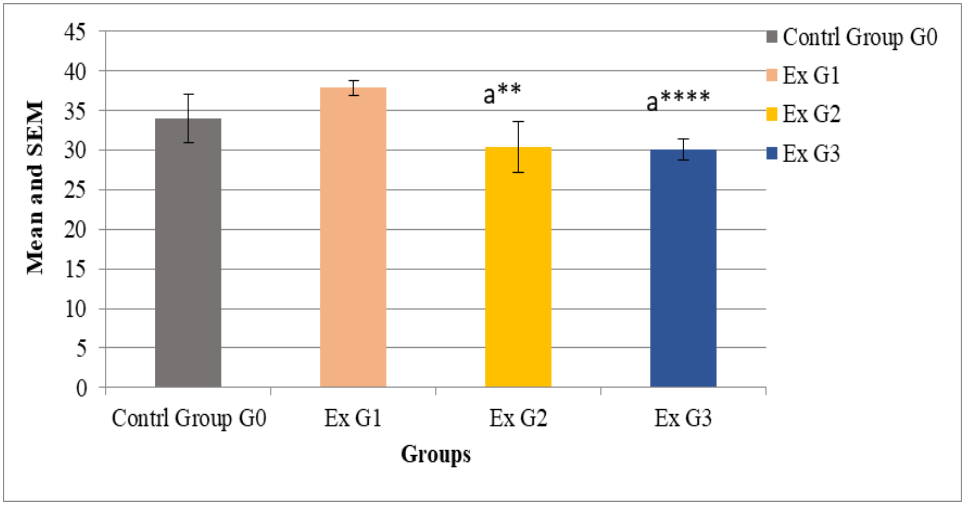


**Figure2.** Morphometric Analysis of Heart Weight of Rabbits between Control Group (Go) and Experimental Groups (G1, G2, G3)

Figure 2 present the comparison of heart weight done between two respective groups when heart weights of control group (Go) compare with experimental groups (G1, G2, G3) no significant difference observed between them. When heart weight of G1 compared with G2 then $p<0.01$ was observed. When heart weight of G1 compared with G3 no significant difference was observed. When heart weight of G2 compared with G3, $p<0.0001$ was observed. Asterisks are representing the significant difference of heart weight among control group (Go) and experimental groups (G1, G2 & G3).

Comparison of CRP level done between two respective groups when CRP level of control group (Go) compared with experimental group (G1), $p<0.001$ was observed. When Go compared with (G2, G3) no significant difference observed between them. When CRP level of G1 compared with G2 then $p<0.001$ was observed. When CRP level of G1 compared with G3 no significant difference was observed. When CRP level of G2 compared with G3, $p<0.001$ was observed. Asterisks are representing the significant difference of CRP level among control (Go) and experimental groups (G1, G2 & G3) (Figure 3).

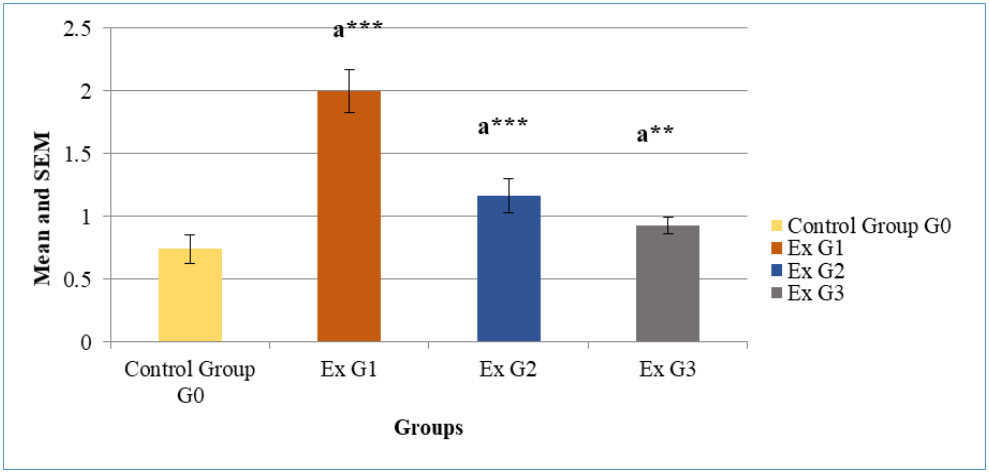


**Figure 3.** Morphometric Analysis of CRP Level of Rabbits between Control Group (Go) and Experimental Groups (G1, G2, G3)

### 3.2. Micropathological Interpretation of Heart Tissues

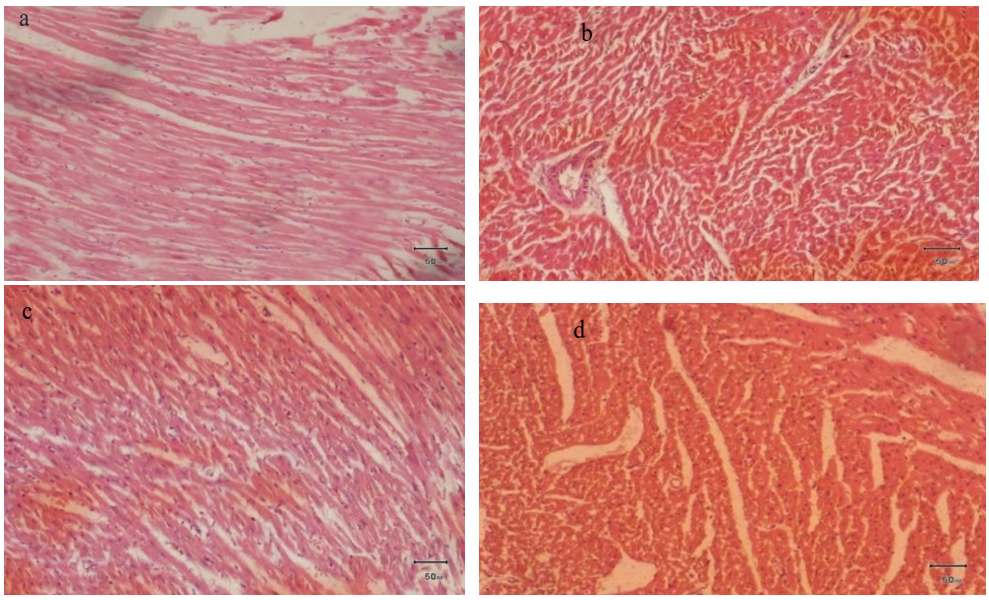


**Figure 4.** Histological Examination of Heart tissues of (a) Control group (Go) (b) Experimental group (G1) (c) Experimental group (G2) (d) Experimental group (G3)

Histological examination of Heart tissues of Control group (Go) fed simple diet showing that heart tissues were almost normal and no changes observed in them (10×10). Histological examination of Heart tissues of Experimental group (G1) fed simple diet and high fat diet indicating mild congestion in the myocardium .H&E (10×10). Histological examination of Heart tissues of Experimental group (G2) fed

simple diet, high fat diet and 5% garlic showing that heart tissues were almost normal and no changes observed in them. H&E (10×10). Histological examination of Heart tissues of Experimental group (G3) fed simple diet, high fat diet and 10% garlic showing that heart tissues were almost normal and no changes observed in them. H&E (10×10)

## 4. DISCUSSION

In the current study it was observed that heart weight of rabbits of experimental group (G1) was more than rabbits of other groups due to the deposition of fat. Increased heart weight indicated that muscles of heart became thick and thickness of heart muscles represented heart defect like cardiomyopathy. Many epidemiological evidences of past few decades has linked the intake of high fat diet and increased risk of cardiovascular diseases. In past studies it was observed that comparable increase in heart weight and cardiac hypertrophy caused by the consumption of high fat diet [11]. it was concluded that experimental group (G1) who fed high fat diet along simple diet had high CRP level as compare to other groups (Go, G2 & G3). In past experiment was conducted on rabbits and it was analyzed that group of rabbits who fed high fat diet had higher CRP levels and triglycerides as compared to the rabbits of other groups. Moreover, their results had shown positive pro-inflammatory status metabolically obese but normal weight characterized by COX-2 expression, increase CRP level but decrease oxidative stress-controlled conditions in early stage of metabolic characteristics of metabolic syndrome [12].

In another study, it was analyzed that *Allium sativum* could significantly improve heart function by the reduction of CRP and by improvement of left ventricular remolding without any side effect [13]. According to the current study it was observed that *Allium sativum* reduced level. So, *Allium sativum* proved to be beneficial herb that play important role in the reduction of CRP level. As *Allium sativum* has many therapeutic properties and unlike other modern drugs it has no side effects. So, it can be used for medicinal purposes. Hence, supplementation of *Allium sativum* can refer to noticeable way of chemical features to health protection due to its strong anti-oxidant properties. In the previous studies [14] investigated that garlic may provide long-term protection against ischemic heart disease reduced CRP level and Lipid profile. Similar study [15] observed that *Allium sativum* diet giving 50% can lower blood sugar and serum lipid levels, rise in HDL concentrations. In the previous studies, observed that cardio-protective properties of garlic *(Allium sativum)* in animal models. In this study involving rats with gentamicin-induced chronic renal failure, garlic extract supplementation led to built renal function, decreased oxidative stress, and normalized cardiac parameters, including $Na^+/K^+$-ATPase action and calcium levels. As the results that garlic's antioxidant properties may play a role in mitigating cardiac pathology associated with renal impairment [16].

### 4.1. Conclusion

The aim of this study was to evaluate the *Allium sativum* effect on CRP and Cardiac Structure in rabbits. In current study *Allium sativum* rescued the CRP level and heart structure in rabbits fed high fat diet . *Allium sativum* supplementation may be a safe adjunct therapy for mitigating heart damage and reducing the risk of cardiovascular disorders, although further studies are needed.

## CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

## DATA AVAILABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

## FUNDING DETAILS

No funding has been received for this research.

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