

Original Article

# Antihyperlipidemic, hypoglycaemic and antioxidative potential of Juglans Regia (walnuts) in an experimental animal model of dietary hyperlipidemia.

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## Abstract

**Background:** Cardiovascular diseases (CVD) are recognized as the world's primary cause of fatality, particularly in the developing countries. Stroke and ischemic heart disease have reportedly been the principal manifestations of vascular disease mainly because of the gradual increase in the incidence of the risk factors. In this aspect, the present study is planned to investigate the cardio-protective potential of Juglans regia (JR) (walnuts) consumption in controlling dyslipidemia. JR has notable therapeutic effects and, it has been used since ancient times for its anti-inflammatory, cardio-protective, and anti-bacterial properties.

**Methodology:** In this study, 32 age-matched females, Wistar albino rats of 5-6 weeks weighing 200 g, were selected and equally divided into four experimental groups. Group I was used as control, Group II was positively induced hyperlipidemia through a high-fat diet; Group III animals were fed normal rat chow along with 153 mg JR powder, and Group IV animals have also induced hyperlipidemia through a high-fat diet incorporated with 153 mg JR powder/Kg for 15 days. Bodyweight changes along with daily diet consumption were measured on alternate days.

**Results:** In comparison to the control group, animals fed with a high-fat diet (Group II) showed a non-significant increase in plasma lipid profile, total cholesterol (TC), triglycerides (TG), and plasma high-density lipoprotein (HDL) levels. Group III and IV animals, after walnut powder supplementation, showed a significant decrease in lipid profile change in very Low-Density Lipoprotein levels (VLDL) of  $10.3 \pm 0.60$  mg/dl and  $12.27 \pm 0.94$  mg/dl together with nephron-protective & reno-protective enzymatic alterations. Histological analysis revealed signs of inflammation in high fat treated groups while well-maintained cardiac tissues within walnut treated groups, respectively.

**Conclusion:** Daily consumption of Juglans Regia (walnut) in the dosage mentioned earlier is beneficial in protectively modulating dyslipidemia, hyperglycemic, and oxidative tissue potential of high-fat diet consumption in an experimental animal model.

## Keywords

Juglans Regia, Walnut, Hyperlipidemia, Hyperglycemia, Cardioprotective Antioxidant Potential.



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## Introduction

Cardiovascular disease has been a significant concern in the developing world, and it is recognized as the world's primary cause of fatality<sup>1,2</sup>. Over 35 million deaths occur annually, 80% are ascribed to chronic diseases similar to the deaths from CVD in developing nations. Alteration in the cardiac disease pattern globally indicates interaction between environmental influence and genetic predisposition, secondary to industrialization, urbanization, and other higher risk factors. Stroke and ischemic heart disease have been reportedly found as the primary cause of CVD in low-income countries compared to high-income countries<sup>3</sup>. Widely known risk features that lead to the development of CVD are sedentary lifestyle, tobacco abuse, increased blood sugar and blood pressure levels, obesity, and high cholesterol. Such factors can be controlled to reduce significantly CVD progression and severity of the condition<sup>4</sup>.

Walnut is the essential temperate nut that grows worldwide<sup>5</sup>. Walnut kernel is a crucial source of essential nutrients such as proteins, fats, vitamins, minerals, and polyphenols that make its nutritional requirement crucial for humans. Walnuts are abundant sources of essential dietary fatty acids like omega-6 and omega-3 polyunsaturated fatty acids. Moreover, these nuts contain essential nutrients for maintaining a healthy lifestyle<sup>6,7</sup>. In contrast to other tree nuts, walnuts have been ranked the highest in "Total radical-trapping antioxidant parameter (TRAP)," "ferric reducing antioxidant power (FRAP)," as well as in the "trolox equivalent antioxidant capacity (TEAC)" assays respectively<sup>8</sup>. Nutritional advantages of walnuts, in general, are primarily associated with high omega-3 fatty acid and some saturated fatty acid content along with positive effects on lipid profiles. Furthermore, due to the higher nutritional content, walnuts have a beneficial effect on the antioxidant structures. They reduce lipid and lipoprotein-mediated oxidative damage, ultimately protecting against atherosclerotic plaque formations<sup>9</sup>.

The current study is designed to investigate the efficacy of daily walnut powder consumption in

controlling the progression of the high-fat diet-induced phenomenon of atherosclerosis in an experimental animal model of dietary hyperlipidemia.

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## Methodology

### Animal model & housing

For this study, thirty-two female Wistar albino rats (*Rattus norvegicus*) weighing 200g and 5-6 weeks of age were used. Animals were bought from the International Center for Chemical and Biological Sciences (ICCBS), Karachi.

Before the experimentation, animal models were acclimatized for a week at the animal housing facility of the Department of Physiology, University of Karachi. Animals were kept in specialized individual cages with proper clean bedding with specified temperature and light/dark cycles (25-30°C, 12-h alternating light/dark cycle). Bodyweight changes along with daily diet consumption were measured on alternate days.

### Diet Preparation

#### a. Preparation of Normal Rat Chow Diet:

For the preparation of a normal rat chow diet, equivalent amounts of "corn flour," "barley flour," "gram flour," and "wheat flour" were mixed with table salt and oil as needed. Small pellets were formed by adding water to this mixture, and after being oven-dried, the pellets were stored in containers at 4°C till further use.

#### b. Preparation of High Fat Diet (HFD):

To prepare "HFD," 40g liquefied cow fat was added to the ingredients as mentioned above in the normal lab diet. These ingredients were then mixed to form small pellets of about 12g. Approximately fifty percent of the calories consumed every day during this diet were resultant from the fat filling incorporated in it<sup>10</sup>.

#### c. Preparation of Walnuts powder:

For this study, whole walnuts without shells were used. These were purchased from a local mart situated in Karachi, Pakistan. Walnuts were ground

in an electric grinding instrument until a fine powder was formed, and this powder was stored in a fresh container till further use<sup>11</sup>.

### **Ethical Considerations**

The procedures undertaken in the present study were as per the ethical guidelines of the National Institute of Health<sup>12</sup>.

### **Research Design**

Following the initial acclimatization period of 1 week, the animals were divided into four experimental groups.

Group I: (Control group) (n= 8) received a standard rat chow diet throughout the 15-day experimental period.

Group II: (Hyperlipidemic group) (n=8) were fed a fat-rich diet exclusively made with melted animal fat incorporated in the diet and were positively induced hyperlipidemia. After the 15 day study, their blood and tissue samples were collected to assess changes in biochemical analysis.

Group III: (JR and normal rat chow diet) (JR treated group) (n=8) this group received 0.153g ground walnuts (JR) with a normal rat diet per rat per day for 15 days.

Group IV: (Juglansregia and High-fat diet) (JR+HFD group) (n= 8) this group received 0.153g walnut (JR) powder with a high-fat diet for 15 days after an initial period of acclimatization.

After the 15 days of experimental study, animals were kept with overnight fasting and anesthetized, and blood was obtained through heart puncture. After drawing blood, animals were dissected for organ removal. Heart, liver, aorta, and kidneys were separated and washed in a chilled solution of saline (0.9% NaCl). These organs were then kept at -80 °C till further usage.

### **Biochemical Examination**

Tests were conducted for analyzing biochemical changes in plasma lipid profile, total protein, glucose, urea, creatinine, uric acid, ALT, AST, ALP,

and cardiac tissue antioxidant levels (catalase, superoxide dismutase) through the Global chemical package.

### **Histopathological Examination**

After fixation, the heart tissue was incorporated in 70% alcohol (isopropyl) for 3 hrs, and later, the tissue was dried for being transformed into paraffin blocks. After that, the tissue segments were then deparaffinized with xylene and rehydrated. Consequently, the hematoxylin/Eosin dyes were used to stain the heart tissue segment and examined under a light microscope<sup>13</sup>. These alterations in tissue samples were rated on a scale of zero to four (0= no change, 1= slight, 2= mild, 3= moderate, and 4= severe) following the severity of structural alteration.

### **Statistical Analysis**

In this study, the data is presented as  $\pm$  SEM (standard error of the mean), control, HFD consumed, and herb treated groups were tested for statistically significant differences using one-way ANOVA, and significance was accepted at  $p < 0.05$ . Tukey's HSD test and Dunnett's test were used to compare the herb-treated groups' data with the control group.

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## **Results**

Results indicated a significant increase in the bodyweight was observed among group II rats compared to the control group with dietary fat supplementation for 15 days. Walnut extract treatment caused a significant decrease in body weight ( $p < 0.005$ ) in comparison to the HFD group. Walnut (JR) extract treatment reduced the body weight in JR+HFD rats compared to HFD groups i.e.  $187.91 \pm 2.69$  kg due to less diet intake.

Plasma levels of LDL-C ( $p < 0.05$ ), VLDL-C ( $p < 0.05$ ), and the atherogenic index ( $p < 0.001$ ) were found to be significantly higher in the HDF group after supplying a fat-rich diet for 15 days in contrast to the control group. Walnut supplementation for 15 days in the JR+HFD group resulted in a significant ( $p < 0.05$ ) decrease in the plasma LDL-C, VLDL-C ( $p < 0.005$ ), and atherogenic index ( $p < 0.005$ ) compared to the HFD group. In the JR treated

group animals, plasma LDL-C, VLDL-C levels, and atherogenic index were significantly low ( $p < 0.05$ ), maintaining a close correlation to baseline values of the control group. Plasma HDL-C levels were significantly ( $p < 0.005$ ) decreased following a fat-rich diet in animals of the HFD group compared to the control group fed a normal rat chow diet. Meanwhile, walnuts extract in animals of the JR+HFD group non-significantly increased plasma HDL-C levels compared to the HFD group. However, this increase was higher than HDL-C levels found in the control group ( $p < 0.01$ ). In the JR group, walnut supplementation regulates plasma

HDL-C levels close to the control group and higher ( $P < 0.005$ ) than the HFD group (Table 1).

The serum TC and TG levels were considerably ( $p < 0.05$ ) higher following fat-rich diet supplementation in rats of the HFD group. Walnut extract incorporation for 15 days within rats of the JR+HFD group decreased the TC and TG rate notably ( $p < 0.005$ ) in comparison to HFD and control group. Meanwhile, in the JG treated group, TC and TG concentrations were non-significantly reduced compared to the HFD group.

**Table 1: Changes observed in body weight, lipid profile among the rats of the compared groups.**

Parameters	Control (n=8)	Hyperlipidemic (n=8)	JR+Normal rat chow (n=8)	JR+ High Fat Diet (n=8)
<b>BW (g)</b>	129.33 ± 8.49	212.167±5.510 <sup>***</sup>	199.71±5.876 <sup>**/*</sup>	187.91±2.693 <sup>***/*</sup>
<b>TC (mg/dl)</b>	100.437±3.58	161.43±27.12 <sup>NS</sup>	114.56±13.35 <sup>NS/*</sup>	69.37±63.54 <sup>NS/NS</sup>
<b>TG (mg/dl)</b>	88.61±3.41	131.04±17.99 <sup>NS</sup>	51.43±2.984 <sup>*/***</sup>	61.33±4.738 <sup>NS/*NS</sup>
<b>HDL-C (mg/dl)</b>	33.22±0.94	24.45±1.167 <sup>*</sup>	26.4±2.83 <sup>NS/NS</sup>	43.18±6.657 <sup>NS/NS/NS</sup>
<b>LDL-C (mg/dl)</b>	49.48±2.274	110.77±27.055 <sup>NS</sup>	77.87±14.93 <sup>NS/*</sup>	92.73±9.56 <sup>NS/*/**</sup>
<b>VLDL-C (mg/dl)</b>	17.72±0.683	26.20±3.59 <sup>NS</sup>	10.3±0.602 <sup>***/****</sup>	12.27±0.94 <sup>*/***/*</sup>
<b>AIP</b>	0.425±0.012	0.709±0.072 <sup>***</sup>	0.306±0.073 <sup>*/***</sup>	0.176±0.072 <sup>*/***/*</sup>

The values mentioned above are represented as Mean ± SEM.

BW= Bodyweight, TC= Total cholesterol, TG= Triglyceride, HDLC = High-Density Lipoprotein, LDL= Low-Density Lipoprotein, VLDL= Very Low-Density Lipoprotein. The significant differences between experimented groups by t-test are: \* $p < 0.05$ , \*\* $p < 0.01$ : \*\*\* $p < 0.005$ . NS results represent non-significant values compared with control/compared with the hyperlipidemic group, JR+Normal rat chow, and JR+High Fat Diet group.

The estimated change in liver enzyme levels of different animal groups was tested, and the results are described in table 2. Administration of a fat-rich diet for 15 days resulted in high serum ALT, AST and ALP values significantly ( $p < 0.005$ ) in animals of the HFD group (Table 2). Following the walnut extract supplementation for 15 days, a significant decline ( $p < 0.05$ ) in Serum ALT, AST and ALP in animals of walnut + high fat diet (group IV) and walnut + normal rat chow (group III) was noted in comparison to group II (Table 2). Nevertheless, this decrease in levels was still higher in contrast to the values of control animals. Transformation in ALT, AST and ALP levels in the JR group were not much apparent ( $p > 0.05$ ) in contrast to control animals.

**Table 2: Changes in Liver Enzymes Levels in among the rats of the compared groups.**

Parameters	Control (n=8)	Hyperlipidemic (n=8)	JR + Normal rat chow (n=8)	JR+ High Fat Diet (n=8)
<b>AST U/L</b>	34.52 ± 2.30	74.04± 2.35 <sup>NS</sup>	49.43±9.68 <sup>NS/NS</sup>	50.62± 5.34 <sup>NS/NS/**</sup>
<b>ALT U/L</b>	25.8±1.393	61.65±2.06 <sup>NS</sup>	26.63±2.28 <sup>NS/NS</sup>	49.96± 2.302 <sup>NS/NS/NS</sup>
<b>ALP (U/L)</b>	55.22±2.69	93.12±2.581 <sup>***</sup>	75.57±9.28 <sup>NS/NS</sup>	57.6±4.093 <sup>NS/NS/**</sup>

The values mentioned above are represented as Mean ± SEM.

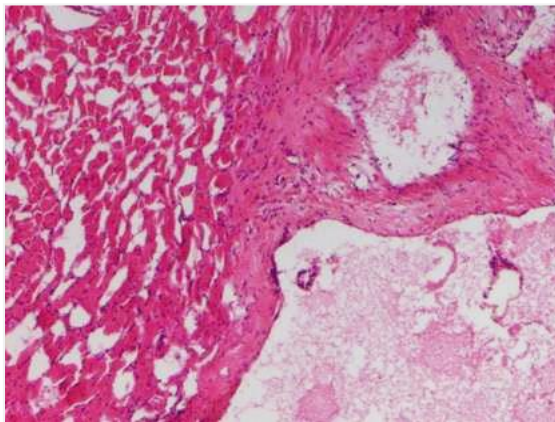
The significant differences between experimented groups by t-test are: \*p <0.05, \*\*p<0.01: \*\*\*p<0.005. NS results represent non-significant values compared with control/compared with the hyperlipidemic group, JR+Normal rat chow, and JR+High Fat Diet group.

Histological Examination of heart tissues correlated with the results retrieved from the biochemical analysis. Tissue sections of the HFD group showed increased lymphocytic infiltration in myocardial walls associated with 15 days of fat-rich diet supplementation in these animals. Walnut extract supplementation tends to manage these changes and alterations in animals of the JR+HFD group. Furthermore, the animal group receiving only walnut extract in addition to a normal lab chow diet showed preserved cardiac wall histology (Table 3).

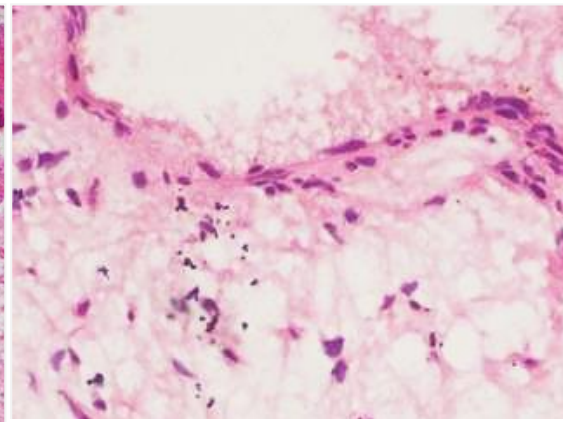
**Table 3: Histopathological Examination of Heart tissue in control, hyperlipidemia, walnut treated (JR+Normal rat chow) and walnut with high-fat diet (JR+ HFD).**

Variables	Control (n=8)	Hyperlipidemic (n=8)	JR + Normal rat chow (n=8)	JR+ High Fat Diet (n=8)
<b>Organ Appearance</b>				
<b>Color</b>	Brownish Red	Brownish Red	Brownish Red	Brownish Red
<b>Texture</b>	Smooth	Smooth	Smooth	Smooth
<b>Lymphocytic Infiltration</b>	-	+2	-	+1
<b>Foam Cells</b>	-	-	-	-

Scale: 0: no distinguishable, +1: slight localized impairment, +2: mild localized impairment, +3: moderate localized impairment, and +4: severe localized impairment

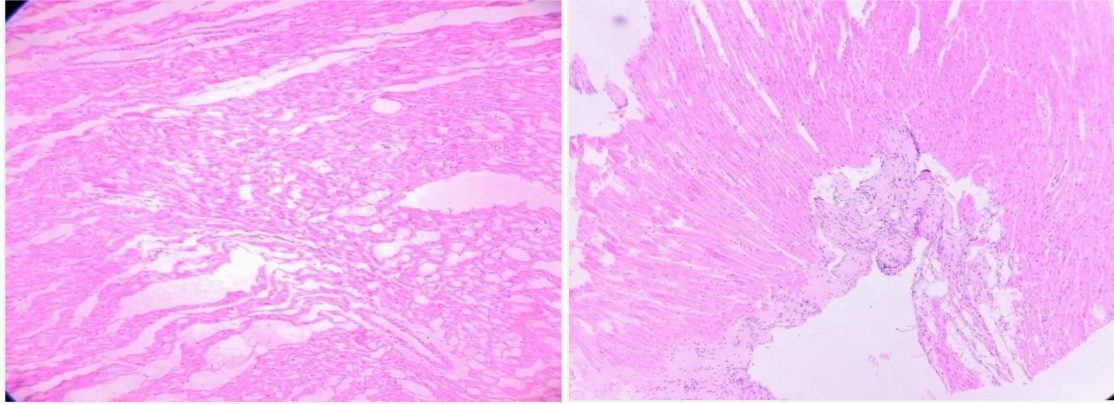


**A. Normal Heart Histology in control animals of group I**



**B. Histopathological changes in the hyperlipidemic group (group II)**





**Figure 1. Histopathological changes in heart section of JR+Normal rat chow (Group III) and JR+HFD group (group IV) with walnut extract (Juglans regia).** (A) Heart morphology of control group displayed. (B) Histopathological changes in heart sections from animals of HFD group. Inflammatory cell infiltrates present. (C) Histopathological changes in heart section from JR group with minimal inflammation. (D) Histopathological changes in heart section from JR+HFD group with visible Focal cartilage & lymphoid aggregates.

## Discussion

Globally, due to extensive changes in living standards throughout the past 10 years, society's inclination towards natural productivity and a healthy lifestyle has now shifted to processed food and a sedentary way of life<sup>14</sup>. The risk features of cardiac disorders have increased with such harmful lifestyle factors with increased smoking. Similarly, the WHO has highlighted CVD as the main cause of mortalities worldwide, causing greater than 30% of all fatalities globally<sup>15</sup>.

The *Juglans* genus, i.e., the Juglandaceae, consists of various species, broadly dispersed worldwide. An important member of this genus is the *Juglans regia*L, commonly known as the walnut tree present in temperate regions and grown for industrial purposes all over the European region, north-Africa, US, South-west America as well as Asia<sup>16</sup>. Among the effective components, walnuts (*JuglansRegia*) contain high levels of ellagic acid, which is a polyphenol, and other components like vitamin E, fibers, and essential fatty acids<sup>17</sup>. In the present study, the 0.153 g of walnut powder in 200gms body weight caused a positive increase in the bodyweight of the group III and IV rats due to the presence of dietary fatty acids like omega-3 and omega-6 Polyunsaturated fatty acids (PUFAs) in walnut.

Being rich in PUFAs, walnuts significantly decrease the threat of cardiac disorders by reducing the total cholesterol and Low-density lipoprotein cholesterol levels while increasing the high-density lipoprotein cholesterol levels. Due to this beneficial property, earlier, nuts have been denoted as the effective anti-atherosclerotic impact of walnuts in human beings<sup>18</sup>. From our study, the results indicated that the administration of walnuts keeps the plasma TC and TG levels balanced at a moderate level. In addition, the walnut supplementation significantly decreased the LDL-Cholesterol and VLDL-cholesterol status in walnut-treated groups (group III & group IV) compared to control and hyperlipidemic groups. Similar results have been deduced from a previous systemic review by Xiao et al.<sup>19</sup> that represented enhanced endothelial functioning mediated by the administration of walnuts. Another study confirmed the anti-hypertensive effect mediated by walnuts in hypertensive rats due to tannic acid in walnuts<sup>20</sup>. The organic antioxidant of walnut extracts, namely vitamin E (tocopherol), is the most impactful in protecting possible CVDs through its antioxidant activity. It is worth mentioning that the essential component of walnuts is polyphenols, and it possesses anti-atherogenic, anti-angiogenic, and antioxidative effects<sup>21,22</sup>. Results from our study indicated that the walnut diet caused a significant

( $P < 0.05$ ) decrease in the liver enzyme levels (i.e., AST, ALT, and ALP) in comparison to the group II hyperlipidemic rats. Our results were continuous with the previous study conducted by Perez-Meseguer et al.<sup>23</sup>, which was the first study to report the nephroprotective potential of Juglans. Mollisto treats the renal injury induced by ischemia-reperfusion. Another study conducted on walnuts confirmed that the hepatoprotective impact of the walnut extract was beneficial against liver damage caused by CCl<sub>4</sub> in rats<sup>24</sup>.

Irrespective of the detailed findings, the study had certain limitations, such as the limited finance to conduct the tissue sampling. With this regard, the tissue sampling was restricted to heart tissue testing, while the liver and kidney tissue samples were excluded, which may have provided valuable results. Lastly, for future studies, it is recommended to further build upon this study and experiment with both sexes in animals. Including male and female animal models can be beneficial for applying research-derived knowledge. This notion has been addressed by Clayton<sup>25</sup> in their research, who have emphasized the significance of sex as a biological variable.

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## Conclusion

In light of the results, it is concluded that the entire 15 days experimental period with walnut supplementation was associated with positive effects on cardiac health and the nephron and hepatic status of the Wistar rats. Therefore, this research revealed the significant anti-hyperlipidemic and antioxidant effects of walnuts. In this prospect, it can be assumed that the overall consumption of walnuts can be beneficial and may potentially limit and/or delay the pathological changes occurring in the body due to cardiovascular diseases. Nonetheless, further researches in this area are also necessary to support the findings of this study.

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## Conflicts of Interest

The authors have declared that no competing interests exist.

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