Ameliorate Effect of Ajwa (*Phoenix dactylifera*) on C-Reactive Protein, Lipid Profile and Cardiac Histopathology in Rabbits

Aisha Saleem\textsuperscript{a*}, Irum Naureen\textsuperscript{b}, Aadab Akhtar\textsuperscript{c}, Gulnaz Tasleem\textsuperscript{d}, Sara Chaudhry\textsuperscript{e}, Attique Nawaz\textsuperscript{f}, Sabeen Ijaz\textsuperscript{g}, Muhammad Naeem\textsuperscript{h}, Zaib ul Nisa\textsuperscript{i}

\textsuperscript{a,de,ifi} M.Phil Researcher School of Zoology, Minhaj University Lahore  
\textsuperscript{b} Assistant professor, School of Zoology Minhaj University Lahore  
\textsuperscript{c} GC Women university Sialkot  
\textsuperscript{d} Centre of Applied molecular Biology and Forensic sciences  
\textsuperscript{e} Institute of Research and Information Mirpur AJK, Pakistan  
\textsuperscript{a} Email: aishasaleem160@gmail.com  
\textsuperscript{b} Email: dr.irunmaureen@mul.edu.pk  
\textsuperscript{c} Email: aadabakhtar018@gmail.com  
\textsuperscript{d} Email: gulnatzasleem65@gmail.com  
\textsuperscript{e} Email: schaudhry718@gmail.com  
\textsuperscript{f} Email: muhammadattique7890@gmail.com  
\textsuperscript{g} Email: sabeenmughal26@gmail.com  
\textsuperscript{h} Email: physiologycardio@gmail.com  
\textsuperscript{i} Email: zees7486@gmail.com

Abstract

**Background:** High fat diet causes oxidation of accumulated fat cells which leads to Atherosclerosis. The inflammatory marker C-reactive protein has recently been identified as an independent predictor of future coronary heart disease. The inflammatory marker C-reactive protein has recently been identified as an independent predictor of future coronary heart disease. Present study evaluated the cardioprotective role of Ajwa seed paste on lipid profile and C-reactive protein (CRP) level in rabbits. **Methodology:** 7 months old rabbits (n=15) for 30 days were kept in animal house school under standard laboratory conditions. Rabbits were divided into three groups (n=5). Group 1 served as control group fed basal diet, group 2 fed high fat diet, group 3 fed high fat diet supplemented with 6gm ajwa seed paste. Feed consumption ratio was determined in all groups on daily basis. **Results:** Feed consumption ratio showed significant (P>0.05) decrease in group 2 and 3 fed high fat diet. In this study, lipid profile (cholesterol, triglyceride, LDL and VLDL) values were increased in group 2 compared to control group. It was found that significant (P>0.05) decrease in the level of bad cholesterol (LDL, VLDL) in group 3 (fed ajwa seed supplemented diet).
HDL value showed significant (P>0.05) increase in group 3. HDL is good cholesterol and increased the level of HDL protect against the stroke and heart attack. and increase the level of good cholesterol (HDL). HDL takes the cholesterol from the body and back to liver where liver removes the bad cholesterol. The value of CRP is higher in group 2 due to the consumption of HFD and CRP level in group 3 showed significant (P>0.05) reduction.

Conclusion: It is concluded from present study that ajwa seed paste is beneficial for heart health.

Keywords: Cardiovascular diseases; Lipid profile; hs-CRP; CRP; ELISA.

1. Introduction

Cardiovascular diseases are group of disorders of heart and blood vessels. Cardiovascular diseases caused by some factors such as unhealthy diet, high blood pressure, smoking, high cholesterol and other lifestyles while sometimes they caused by family history, gender, age and genetic Reference [1]. There are four main types of cardiovascular diseases which are coronary heart disease, cerebrovascular disease, peripheral arterial disease and congenital disease Reference [2]. Ajwa has a great option for cardiovascular disease. Ajwa have own promising and nutritional value compounds for heart diseases. In Islamic world, it's known as king of its species. 450 types of dates is cultivated in Saudi Arab but ajwa is most expensive due to its medical properties and its nutrition values Reference [3]. There are two types of ajwa dates. One is soft and second is dry ajwa [4]. Ajwa dates are most important due to their high nutritional value like vitamins, carbohydrates, fats fibers and protein. It also consists of phytochemicals like glycosides, phytosterols and polyphenols [5]. These phytochemicals improved the anti-oxidation, anti-inflammatory, cardioprotective, and lipid profile [6]. Ajwa have 314 kcal of energy, which is more than other dates. Ajwa have sugar content is monosaccharide about 77% in which 0.5% sucrose, 34% glucose and 25.6% fructose. Minerals content about 3% which is more than ordinary dates which are only 1.5 to 2.7%. Calcium amount is higher around about 1.22 grams. These all properties are making the jawah different and popular than the other dates nutritional and medical value is high Reference [7]. They have 23 types of amino acids and 6 vitamins including A, B1, B2, C nicotinic acid and riboflavin [8-9]. There are also abundant of minerals like calcium, zinc, potassium, magnesium, copper and iron and other compounds like Carotenoids, flavonoids, phenolic phytosterols and procynidines. In ajwa, niacin decreases the level of low density lipoprotein and cholesterol in blood [10]. C-reactive protein comes from the liver. C-reactive protein test measure the amount of protein in blood. C-reactive protein level raised when inflammation in the body [11].

Figure 1: Ajwa dates [12]
First time CRP substance found in the serum of patient that effected by the pneumococcus. C is the abbreviation of carbohydrate antigen [13]. CRP can be a casual factor as well as a maker of inflammation. The fact is that CRP a stable protein. C-reactive protein elevated several times folds within the 24 – 48 hours in an inflammatory disease, injury tissues. It has been shown that high CRP levels can predict the long term risk of myocardial infarction, stroke, peripheral arterial disease and sudden cardiac death in apparently healthy subjects Reference [14].

2. Materials and Methods

The Experiment was performed at Minhaj University Lahore to examine the Ajwa (Phoenix dactylifera) Ameliorate the Effect of High Fat diet on C - reactive protein, Lipid Profile and Cardiac Histopathology in Rabbits.

2.1 Collection of blood after animal sacrifices

Ethical approval was taken prior to study from ethical approval committee of Minhaj University Lahore. 12 rabbits were bought from University of Veterinary and Animal Sciences, Lahore. The experiment of 30 days the mean body weight of each group of rabbit was recorded. Group 1 (control group) received simple feed 200mg was given simple feed (pellets) to group 1 of rabbits. Group 2 (Experimental group 1) received simple feed with coconut oil 200mg was given 180mg simple feed (pellets) and 20ml coconut oil to group 2 rabbits. Group 3 (Experimental group 2) received simple feed, coconut oil and ajwa paste 200mg was given simple feed (pellets) 12mg ajwa paste and 20ml coconut oil Reference [15].

Figure2: Pallets with HFD
2.2 Blood Sample Collection

Blood samples were collected from the neck of every rabbit with help of test tubes and transfer to centrifuge tube. The blood samples centrifuge 3000rpm at 15 minutes. The blood was clotted and sera were separated. The samples were examined with further analysis of lipid profile and C-reactive protein. Lipid profile (cholesterol, LDL, HDL, triglycerol) will be analyzed by automatic analyzer. C-reactive protein in serum will be analyzed by ELISA method.

2.3 Histology of Heart tissue

Histology of heart is important for diagnosis purpose. Obtain the cardiac tissue from the heart. During the endomyocardial biopsy procedure, 1-2mm endocardium and myocardium obtained from the heart of rabbits. The peripheral proximity of the biopsy to the venous inlet and a thicker wall than the atrium make the right ventricle an ideal site for biopsy. To preserve the cardiac sample and placed into the formalin. This preserved sample placed into the cassettes. After that, preserved sample embedded in paraffin wax. After obtained the thinly sliced from the sample and kept onto the glass slide. Eosin and hematoxylin is a stain to visualization of heart tissue under the microscope.

2.4 Statistical analysis

Data Analysis was done by using graph pad prism version 5. To find out the values of mean and standard error mean (SEM) and P-value by using t-test. The data was considered significant when the P-value was less than 0.05, (p ≤ 0.05). Mean ± Standard error mean was used to express the values. P-value of each test show possible significance of each variable.

3. Results

In this study 12 rabbits were used for experiment and examined the Ajwa (Phoenix dactylifera) Effect of High Fat diet on C - reactive protein, Lipid Profile and Cardiac Histopathology in Rabbits and following parameters were compared to examine the statistically identifying similarities and differences among them. The initial and final body weight recorded. After test of lipid profile (cholesterol, LDL, HDL, VLDL and triglycerol) different values gained from three groups (control group, EG1 and EG2).

Table 1: Values of feed consumption (Mean and SEM) of control and experiment groups

<table>
<thead>
<tr>
<th></th>
<th>1st Week</th>
<th>2nd Week</th>
<th>3rd Week</th>
<th>4th Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>EG1</td>
<td>99.75±19.87 a***</td>
<td>21.5±2.87 a*</td>
<td>12.5±12.5 a**</td>
<td>0±0</td>
</tr>
<tr>
<td>EG2</td>
<td>74.25±11.82 a***</td>
<td>44±9.05 a**</td>
<td>37.5±9.68 b*</td>
<td>0±0</td>
</tr>
</tbody>
</table>
**Control group:** pellets, **EG1:** Pellets + 10% coconut oil, **EG2:** Pellets + 10% coconut oil + 6gm ajwa paste. 

P>0.05, P<0.05*, P<0.01**, P<0.001***, P<0.0001****

**Figure 4:** Ajwa (*Phoenix dactylifera*) Ameliorate the Effect of High Fat diet on C-reactive protein, Lipid Profile and Cardiac Histopathology in Rabbits. Values of feed consumption (Mean and SEM) of control and experiment group was significantly increased.

**Figure 5:** Ajwa (*Phoenix dactylifera*) Ameliorate the Effect of High Fat diet on C-reactive protein, Lipid Profile and Cardiac Histopathology in Rabbits. Values of feed consumption (Mean and SEM) of control and experiment group in week 2 and week 3 was significantly increased.

**Table 2:** Initial weight final weight and weight gain (kg) (Mean and SEM) of control and experiment groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>EG1</th>
<th>EG2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial weight</strong></td>
<td>1.161 ± 0.040</td>
<td>205.96 ± 204.67</td>
<td>747.5 ± 39.29</td>
</tr>
<tr>
<td><strong>Final weight</strong></td>
<td>1.34 ± 0.0225</td>
<td>1.277 ± 0.7696</td>
<td>1.33 ± 0.134</td>
</tr>
<tr>
<td><strong>Weight gain</strong></td>
<td>0.181 ± 0.025</td>
<td>0.125 ± 0.053 a*</td>
<td>0.583 ± 0.149 b*</td>
</tr>
</tbody>
</table>

**Control group:** pellets, **EG1:** Pellets + 10% coconut oil, **EG2:** Pellets + 10% coconut oil + 6gm ajwa paste. 

P>0.05, P<0.05*, P<0.01**, P<0.001***, P<0.0001****
Figure 6: The graph shows the significance between the control group and EG1, EG1 and EG2

Table 3: Values of heart weight (gm) (Mean and SEM) of control and experiment groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>EG1</th>
<th>EG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight</td>
<td>2.44 ± 0.159</td>
<td>3.432 ± 0.229 a*</td>
<td>2.6 ± 0.165b*</td>
</tr>
</tbody>
</table>

Control group: pellets, EG1: Pellets + 10% coconut oil, EG2: Pellets + 10% coconut oil + 6gm ajwa paste P<0.05*(Values are expressed as Mean and SEM) P>0.05, P<0.05*, P<0.01**, P<0.001***, P<0.0001****

Figure 7: The graph shows the significance between the control group and EG1, EG1 and EG2 due to HFD P<0.05* (Values are expressed as Mean and SEM)

Table 4: Values of Cholesterol (md/dl) (Mean and SEM) of control and experiment groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>EG1</th>
<th>EG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>64 ± 2.76</td>
<td>83.25 ± 4.71 a*</td>
<td>61 ± 3.24 b*</td>
</tr>
</tbody>
</table>

Control group: pellets, EG1: Pellets + 10% coconut oil, EG2: Pellets + 10% coconut oil + 6gm ajwa paste P<0.05*(Values are expressed as Mean and SEM)
Figure 8: The graph show the significance between the control group and EG1 and EG1 and EG2. The value of cholesterol increased in EG1 due to the consumption of oil. P<0.05*

Table 5: Values of triglycerol (mg/dl) (Mean and SEM) of control and experiment groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>EG1</th>
<th>EG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerol</td>
<td>154.25 ±14.77</td>
<td>225 ± 15.32 a*</td>
<td>180 ± 8.57</td>
</tr>
</tbody>
</table>

Control group: pellets, EG1: Pellets + 10% coconut oil, EG2: Pellets + 10% coconut oil + 6gm ajwa paste P<0.05*

Figure 9: The graph show the significance between the control group and EG1. Because the EG1 taken the coconut oil. P<0.05*

Table 6: Values of HDL (mg/dl) (Mean and SEM) of control group and experiment groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>EG1</th>
<th>EG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL</td>
<td>17.27 ± 3.75</td>
<td>29.75 ± 2.01 a*</td>
<td>37.25 ± 1.108 a***</td>
</tr>
</tbody>
</table>

Control group: pellets, EG1: Pellets + 10% coconut oil, EG2: Pellets + 10% coconut oil + 6gm ajwa paste P>0.05, P<0.05*, P<0.001*** (Values are expressed as Mean and S
Figure 10: The graph shows the significance between control group and EG1, EG2. The value of HDL in EG2 increased due to ajwa paste.

Table 7: Values of LDL (mg/dl) (Mean and SEM) of control and experiment groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>EG1</th>
<th>EG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>27.125 ± 4.00</td>
<td>45.25 ± 5.55 a*</td>
<td>33.75 ± 2.98</td>
</tr>
</tbody>
</table>

Control group: pellets no 44, EG1: Pellets no. 44 + 10% coconut oil, EG2: Pellets no 44 + 10% coconut oil + 6gm ajwa paste P<0.05*(Values are expressed as Mean and SEM)

Figure 11: The graph shows the LDL (mg/dl) value of all groups of rabbits the graph shows the significance between the control group and EG1, due to the consumption of high fat diet
Table 8: Values of CRP (mg/l) (Mean and SEM) of control and experiment groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control group</th>
<th>EG1</th>
<th>EG2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>0.222 ± 0.008</td>
<td>6.5 ± 1.5 a**</td>
<td>0.22 ± 0.0081 b**</td>
</tr>
</tbody>
</table>

**Control group:** pellets, **EG1:** Pellets + 10% coconut oil, **EG2:** Pellets + 10% coconut oil + 6gm ajwa paste P<0.01**

![Graph showing significance](image1)

**Figure 12:** The graph shows the significance between control group and EG1 and EG2. The graph shows the CRP (mg/l) value of all groups of rabbits.

**3.1.1 Histopathological examination of rabbits heart tissues**

The histological examinations of rabbit’s heart tissue. Control group with normal diet no fat change in tissue. Experiment group 1 high fat diet fat changing in the tissue seen clearly. EG2 high fat diet with ajwa paste no fat changing in the tissue and similar to control group.

![Histological image](image2)

**Figure 13:** Histological examination of control group. **Magnification:** 10 x 10 **Interpretation:** There is no change in the tissue and no fat change seen. **Black circle:** nucleus **Yellow arrow:** pink substance around nucleus is cytoplasm **Orange arrow:** long cords of myocytes connected by junctionCardiac myocytes arranged in syncytium like pattern.
Figure 14: Histological examination of EG1 (high fat diet). Magnification: 10 x 10 Interpretation: There is fat deposition in the tissue seen very clearly due to consumption of oil. **Black circle:** nucleus  **Yellow circle:** cytoplasm  **Red arrow:** branched cardiac muscle  **Green arrow:** connective tissue layer

Figure 15: Histological examination of EG2 (high fat diet with ajwa paste). Magnification: 10 x 10 Interpretation: no deposition of fat changing in the tissue due to the ajwa paste. **Black arrow:** nucleus  **Green arrow:** cytoplasm  **Yellow arrow:** branched cardiac muscle  **Orange arrow:** long cords of myocytes connected by junction

1. 4. Discussion

The present study was conducted to inspect the ajwa (*Phoenix dactylifera*) ameliorate the effect of HFD on CRP, lipid profile and cardiac histology in rabbits. 12 healthy and same size rabbits were used for the experiment. The body weights of all rabbits were measured. Control group gain weight after consuming simple feed and value was 0.181 ± 0.025, EG1 gain the weight after received the high fat diet and value was 0.125 ± 0.053 and EG2 gain weight after given the high fat diet+ ajwa paste and value is 0.583 ± 0.149. At the end result showed that EG2 gain weight due to high fat diet and EG2 also gain the weight but increased growth performance due to ajwa paste. The heart weight of EG1 is higher than the control group and EG2. EG1 consumed the high fat diet for 30 days. Thickness in heart muscle increased the weight and disease. In the Previous studies showed the intake high fat diet increased the obesity, weight gain and accumulated the oxidation in body. High fat diet produces the cardiovascular diseases Reference [16]. Values are expressed as
Mean and SEM. Asterisk shows the significance between control group and experiment groups.

In the experimental groups received the high fat diet. One experiment group (EG1) given high fat diet and other group (EG2) given the high fat diet with ajwa. Both groups consumed the feed very less in starting weeks than the control group. Coconut oil contains 90% saturated fats and 9% unsaturated fats. Coconut oil also contains medium chain fatty acid (MCFA) [17]. In the present study, lipid profile (cholesterol, triglycerol, LDL and VLDL) values were increased in the experiment group (EG1) than other experiment group (EG2) and control group. EG1 group received the high fat diet (simple feed +coconut oil) for 30 days. So, the values of lipid profile are high. While the EG2 received the simple feed + coconut oil + ajwa paste). So, the values of lipid profile are low.

In the Previous research studies showed that the effect of ajwa on the lipid profile (total, cholesterol, triglycerol, LDL and VLDL) and increased the levels in the blood due to the consumption of high fat diet [18]. HDL value of EG2 is increased due to the ajwa paste. HDL is good cholesterol. HDL takes the cholesterol from the body and back to liver where liver removes the bad cholesterol [19]. The value of CRP is higher in EG1 due to the consumption of HFD. The value of EG2 is normal due to the ajwa paste. Present of supplementation in ajwa stop the antiflammatory and anti-oxidation. In previous studies [20] found that several varieties of date palm fruit exhibit antihyperlipidemic and antihypercholesterolemic effects. Fruit (dates) suspension uses phenolic chemicals to inhibit the breakdown of fat in the liver and has a hypolipidemic effect contained in date fruit, which lower levels of LDL, VLDL, and plasma cholesterol.

In the present study the value of control group is normal due to the simple feed. The value of CRP in control group 0.222 ± 0.008, EG1 value of 65 CRP is 6.5 ± 1.5 and EG2 value is 0.22 ± 0.08. As shown in table 3.8 and graph 12 the values of CRP in control group and EG2 are same. Values are expressed as Mean and SEM. In previous research studies [21] ajwa paste improved the lipid profile and better for CRP level. So, it’s a main part of current study the effect of ajwa paste on the CRP level on heart. In previous studies [22] examined the effects of Ajwa date seed powder on CRP level in diet-induced hyperlipidemic rabbits. The rabbits were selected due to their similarity to human lipid metabolism. After the diet, there was a significant decrease in lipid profile and maintain the level of CRP levels that is a good prognostic factor for atherosclerosis, and the study found that statins do not effectively raise CRP levels.

In the present study histological examinations of rabbit’s heart tissue. Control group with normal diet no fat change in tissue. Experiment group 1 high fat diet fat changing in the tissue seen clearly. EG2 high fat diet with ajwa pastes no fat changing in the tissue and similar to control group in previous studies. In the previous studies Reference [23] by using Ajwa fat deposition in the tissue and cardiac muscle in branched shaped attached with connective tissue layers.

5. Conclusion

This current study showed that rabbits consumed the high fat diet gain the organ weight heart weight, obesity and also showed the fat deposition in heart tissue compare to other groups like EG1. In this study, lipid profile
values were increased in group 2 compared to control group. It was found that significant (P>0.05) decrease in the level of bad cholesterol (LDL, VLDL) in group 3. HDL value showed significant (P>0.05) increase in group 3. HDL protect against the stroke and heart attack and increase the level of good cholesterol (HDL). HDL takes the cholesterol from the body and back to liver where liver removes the bad cholesterol. The value of CRP is higher in group 2 due to the consumption of HFD and CRP level in group 3 showed significant (P>0.05) reduction. So, the current study showed that used of ajwa paste in daily basis control the cardiovascular disease and improve the heart health.

References


