# EFFECT OF AJWA DATE SEED ON LIPID PROFILE OF DIET INDUCED HYPERLIPIDEMIC RABBITS

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## ABSTRACT

**OBJECTIVE:** To evaluate the antihyperlipedemic effect of Ajwa date (*Phoenix dactylifera*) seed on serum lipid levels of diet induced hyperlipidemic rabbits.

**METHODS:** This randomized controlled study of six months' duration was carried out at Postgraduate Medical Institute, Lahore after obtaining permission from institutional ethical committee. Male rabbits, 32 in number weighing between 0.9-1.3 kg were divided into four groups normal control (NC), high fat diet (HFD), HFD+2%Ajwa date seed powder (HFD+2%A) and HFD+4% Ajwa date seed powder (HFD+4%A) having 8 rabbits in each group. Group NC took normal diet while all other groups were given high fat diet for whole 12 weeks. After 4 weeks groups HFD+2%A and HFD+4%A; were given diet containing Ajwa date seed powder 2gm and 4gm per 100 gm of high fat diet respectively for next 8 weeks. Lipid profile was done at 0, 4, 8 and 12 weeks.

**RESULTS:** Serum high density lipoprotein cholesterol (HDL) is increased while serum cholesterol, triglycerides, low density lipoprotein cholesterol (LDL), atherogenic index of plasma and LDL/HDL ratio decreased significantly in both treatment groups. At 8th week HFD+4%A was more effective than HFD+2%A but at 12th week both were equally effective.

**CONCLUSION:** Ajwa date seeds were effective in improving the lipid profile despite high fat diet.

**KEY WORDS:** Diet, High-Fat (MeSH); Dyslipidemias (MeSH); Phoeniceae (MeSH); Ajwa Date Seed (Non-MeSH); Seeds (MeSH); Phoenix dactylifera (MeSH).

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## INTRODUCTION

Dyslipedemia is a metabolic disorder, characterized by increase in total cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride concentrations and a decrease in high-density lipoprotein (HDL) cholesterol concentration in the blood.[1] Low levels of HDL-cholesterol and increase in LDL-cholesterol and triglycerides increase the fat deposition in arteries which is an important cause of ischemic heart disease.[1] Other complications include acute pancreatitis[2] and insulin resistance, which ultimately leads to development of diabetes mellitus.[3] These complications increase morbidity and mortality, which may be reduced by controlling dyslipedemia. Control of lipid level is more important than control of hypertension in prevention of cardiovascular diseases, reducing the risk by 35-40%.[4]

The latest recommendation by Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program is non pharmacologic approach in dyslipidemic individuals including modification in diet and physical activity.[5] It is observed that plant based natural products have minimum side effects and affect multiple targets for preventing and treating hyperlipidemia.[6] In recent years, an explosion of interest in the numerous health benefits of dates has led to many in vitro and animal studies as well as identification and quantification of various classes of phytochemicals. Dates are helpful in the treatment of obesity,[7] atherogenesis and control of diabetes.[8]

Ajwa date seed contains dietary fiber (73.1g/100g), fifteen salts and minerals including calcium, magnesium, potassium and phosphorus in higher concentrations than date flesh.[9,10] It also contains phenolics (3942mg/100g), antioxidants (80400µmol/100g) and carotenoids.[11] High concentration of antioxidants can be very much effective against hyperlipidemia and atherosclerosis.[12] Date flesh has been shown to reduce lipid levels on high fat feeding[13] and as seeds contain more antioxidants, they are expected to have more beneficial effects. Objective of this study was to evaluate the effects of Ajwa date seed powder on lipid profile of hyperlipidemic rabbits.

## METHODS

This experimental study was conducted at Post Graduate Medical Institute, Lahore, Pakistan. Sample collection was done from April to July 2013.

**Animal Selection:** Thirty two (32)

adult healthy male rabbits weighing 0.9-1.3 kg were purchased from local market and kept in cages measuring 45"×27"×24" at room temperature of 20±2°C in a natural light/dark cycle. During the one week period of acclimatization, the rabbits were fed with standard rabbit chow and water ad libitum and were observed for signs of any disease. After acclimatization the rabbits were equally divided into 4 groups by lottery method with mean body weight of 1.0, 1.1, 1.1, and 1.1kg in NC, HFD, HFD+2%A and HFD+4%A respectively. Each group was kept in separate cage.

Group NC: Rabbits fed on normal rabbit chow throughout 12 weeks.

Group HFD: Rabbits received high fat diet for 12 weeks.

Group HFD+2%A: Rabbits received high fat diet for 12 weeks and 2 % Ajwa date seed powder was added after 4 weeks for next 8 weeks. .

Group HFD+4%A: Rabbits received high fat diet for 12 weeks and 4%Ajwa date seed powder was added after 4 weeks for next 8 weeks.

**Preparation of Diet Pellets:** To prepare rabbit chow following ingredients were mixed: Split chick peas (*Cicer arietinumb*) 500grams, dry fodder (*Elusinae coracana*) 500grams, jawar (*Sorghum bicolor*) 500grams and plain flour (*Triticum astivum*) 300 gram used with water to bind all these ingredients in pellet form, dried and kept in refrigerator.

**Preparation of High Fat Diet:** High fat diet contained cholesterol 1g and coconut oil 4ml per 100g of normal rabbit diet.[14] All the ingredients were mixed thoroughly to make diet pellets. Diet was prepared fresh at one week interval and stored in refrigerator.

**Preparation of Ajwa Date Seed Powder:** Ajwa dates were purchased from Aseel date factory of Madina-tul-Munawarra and 2.8kg seeds were taken out of 20kg dates, washed, air dried and finely ground in a powder form and kept in refrigerator. Date seed powder was mixed in high fat diet; 2% and 4% for group HFD+2%A and group HFD+4%A respectively.

**Collection of Blood Samples:** Blood samples were taken at 0, 4, 8 and 12 weeks. Before taking sample animals were deprived of food for 14 hours[15] but had free access to water. One and half (1.5) to 2ml blood sample was drawn from marginal ear vein of each rabbit using 23 gauge needles. Blood was allowed to clot in centrifuge tube at room temperature for one hour and then centrifuged at 3000 revolutions per minute for ten minutes at room temperature to separate serum which was stored at -20°C until analyzed for lipid profile.

**Estimation of Lipid Profile:** Lipid profile was done by enzymatic end point method using commercially available kits (HUMAN Diagnostics, Germany) with spectrophotometer at 546nm wavelength. Serum triglycerides, total cholesterol, high density lipoproteins cholesterol and low density lipoproteins cholesterol were measured. LDL/HDL ratio and atherogenic index of plasma (AIP) were calculated.[16]

**Data Analysis:** After collecting the data it was entered into SPSS 17. To see the significance of outcome in 4 study groups one way ANOVA was applied followed by Tukey's test. Paired sample t-test was used to compare end of study levels with base line within groups. P-value of ≤0.05 was considered significant. Graph Pad Prism 5 was used for graphical presentation of data.

## RESULTS

Serum triglyceride, total cholesterol and LDL-cholesterol were normally distributed, while HDL-cholesterol and its derived parameters LDL/HDL ratio and AIP were not normally distributed. Their baseline values were taken as 100% and change was calculated as percentage of baseline.

Serum triglyceride (TG), total cholesterol, LDL-cholesterol levels, LDL/HDL ratio and AIP increased in all high fat diet (HFD) groups at 4th week. They kept on increasing in group HFD and decreased in group HFD+2% A and HFD+4% A at 8th and 12th week (Figure 1-5). HDL-cholesterol level decreased in all HFD groups at 4th week. It further decreased in group HFD at 8th and 12th week, while increased in group HFD+2% A and HFD+4% (Figure 6).

## DISCUSSION

This study was conducted on diet induced hyperlipidemic rabbits to see the effects of Ajwa date seed powder on lipid profile. Animals selected were rabbits because they have close resemblance with human lipid metabolism. In both species apoB-48 is a major apolipoprotein of chylomicrons and chylomicrons remnants which carry exogenous lipids derived from food. ApoB-100 is a major apolipoprotein of very low density lipoproteins cholesterol (VLDL), intermediate density lipoprotein cholesterol (IDL) and LDL-cholesterol, which are endogenous lipoprotein derived from liver.[17] Only male rabbits were selected to avoid any variation in lipid levels due to gonadal hormones. High fat diet markedly increased blood lipid levels

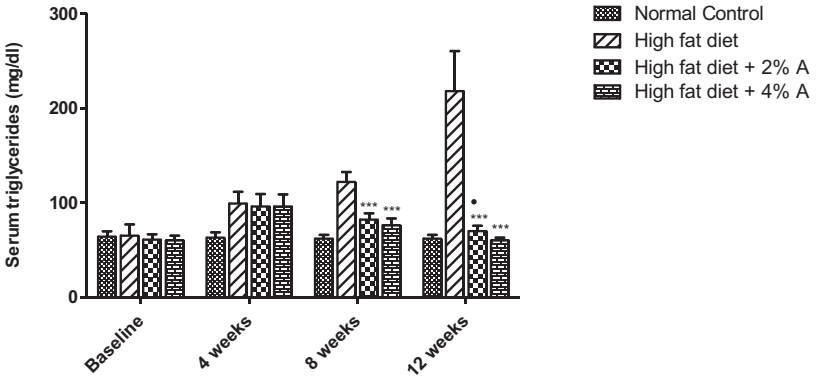


Figure 1: Effect of Ajwa date seed powder on serum triglycerides of control and treated rabbits (mean±SD) (n=8). ***p-value≤0.001vs high fat diet (HFD) group; •p-value ≤0.05 vs baseline

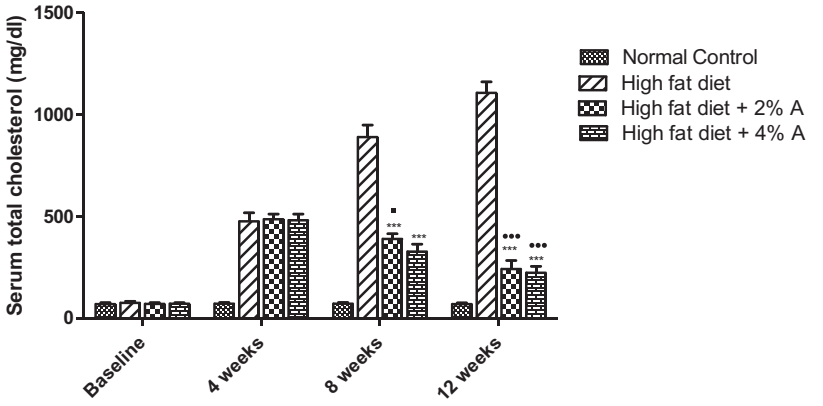


Figure 2: Effect of Ajwa date seed powder on serum total cholesterol of control and treated rabbits (mean±SD) (n=8). ***p-value≤0.001 vs high fat diet (HFD) group; •••p-value≤0.001 vs baseline; ▪p-value≤0.05 vs HFD+4%A group

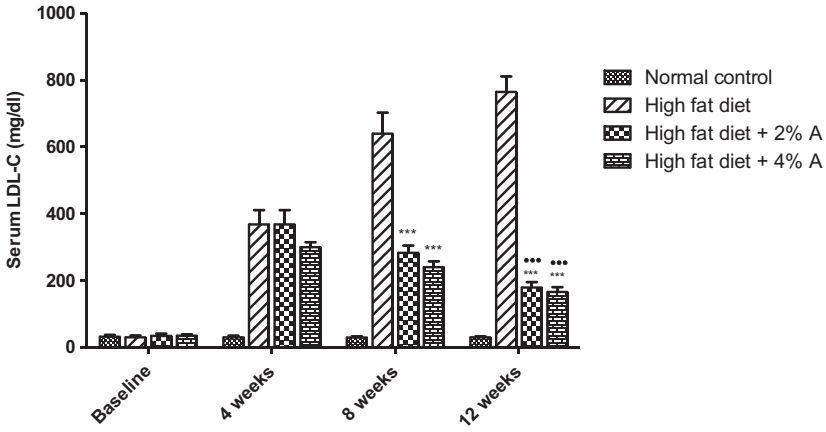


Figure 3: Effect of Ajwa date seed powder on serum low density lipoprotein cholesterol (LDL-C) of control and treated rabbits (mean ± SD) (n=8). ***p-value≤0.001 vs high fat diet (HFD) group; •••p-value≤0.001 vs baseline

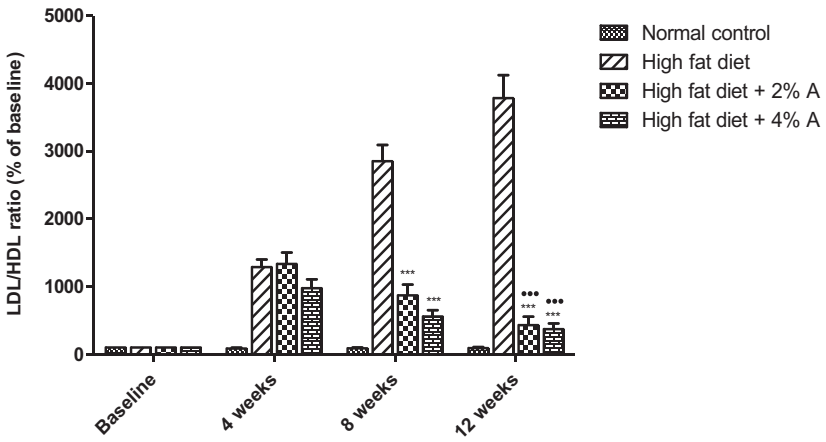


Figure 4: Effect of Ajwa date seed powder on serum LDL/HDL ratio of control and treated rabbits (mean±SD) (n=8). ***p-value≤0.001 vs high fat diet (HFD) group; •••p-value≤0.001 vs baseline

after four weeks. Other studies also demonstrate development of hyperlipidemia in same time period.[18] After the administration of Ajwa date seed powder there was a significant decrease in serum triglyceride, total cholesterol, LDL, LDL/HDL and AIP, while HDL increased in both treated groups.

Decrease in TG level in both treated groups and insignificant difference from baseline in 4% treated group at the end of study was observed. Lowering of TG level is good prognostic factor for atherosclerosis.[19] At the end of study lowering of AIP to baseline in 2% treated group and less than baseline in 4% treated group is an important finding. Atherogenic index of plasma is good predictor of cardiovascular risk even in the presence of co-morbid conditions.[20]

Increase in HDL level above baseline in both treated groups and above NC in 4% treated group is hallmark of the study. Statins, the most commonly used antihyperlipedemic agents, do not raise HDL effectivly.[21] Only niacin has been shown to be effective in this regard [22] but it has low tolerability.

Comparison of both these groups revealed that difference at the 8th week was minimized at 12th week indicating that lower dose for prolonged time was equally effective as high dose. In a study on lipid profile of diabetic rats, seed extract lowered total cholesterol and TG close to normal control with insignificant difference,[23] whereas Ajwa date extract has shown to decrease serum total cholesterol and LDL-cholesterol and increase HDL-cholesterol with no significant effect on triglyceride level in hypercholesterolemic albino rats.[24]

Possible mechanism of action of lowering lipids is the presence of some active constituents of date seed such as antioxidants like polyphones and flavonoids which are highest in Ajwa date seed as compared to other date variety.[24] Increase in antioxidant enzymes like blood glutathione peroxidase, catalase and superoxide dismutase with Ajwa extract[25] strengthens this speculation. Presence of fiber in date seed[9] may also reduce

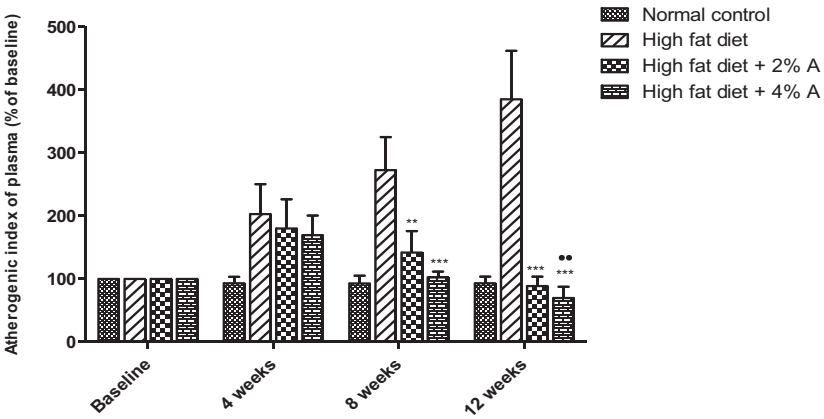


Figure 5: Effect of Ajwa date seed powder on atherogenic index of control and treated rabbits (mean±SD) (n=8). ***p-value≤0.001 vs high fat diet (HFD) group; ••p-value≤0.01 vs baseline

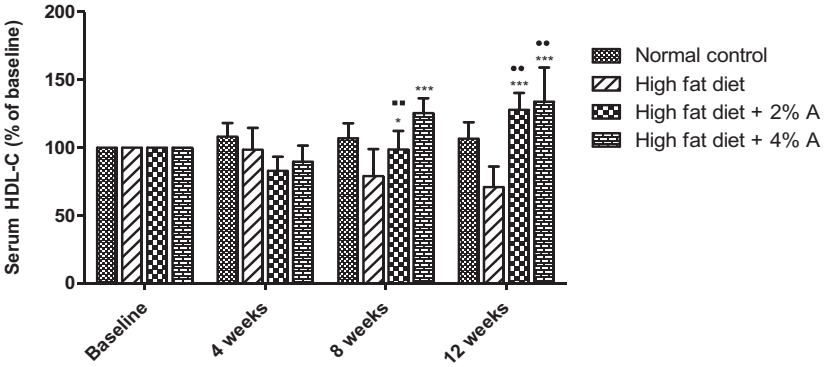


Figure 6: Effect of Ajwa date seed powder on serum high density lipoprotein cholesterol (HDL-C) of control and treated rabbits (mean±SD) (n=8) ***p-value≤0.001 vs high fat diet (HFD) group; ••p-value≤0.01 vs baseline; ▪▪p-value≤0.01 vs HFD+4%A

absorption of dietary fats leading to improvement in lipid profile.

Finally it is summarized that Ajwa date seed had antihyperlipidemic effect on high fat diet fed rabbits. Initially this effect was dose dependent but with continued treatment effect of low and high dose had same beneficial effect. In this study neither a standard drug like statin was used for comparison nor were the atherosclerotic changes evaluated. Further human studies may be conducted to confirm the beneficial effects.

## CONCLUSION

Ajwa date seed powder has significantly improved the lipid profile of diet induced hyperlipidemic rabbits.

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## AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

**ZM:** Concept & study design, acquisition, analysis & interpretation of data, final approval of the version to be published

**SK & NK:** Drafting the manuscript, final approval of the version to be published

**SC:** Critical review, supervision, final approval of the version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

**CONFLICT OF INTEREST**

Authors declared no conflict of interest

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