ORIGINAL ARTICLE
EFFECT OF THREE PAKISTANI DATE-SEED VARIETIES ON LIPID PROFILE OF DIET INDUCED HYPERLIPIDEMIC RABBITS

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Background: Hyperlipidemia is one of the major risk factors of cardiovascular diseases. In addition to current therapeutic strategies, a lot of work is being done on nutraceutical management of this condition. This study was designed to assess the effect of date seed powder on lipid profile of diet induced hyperlipidemic rabbits. Methods: Thirty male rabbits were divided into five groups, having six animals in each. One group was given normal rabbit chow throughout the study period of eight weeks. The remaining four groups were fed high fat diet (4% coconut oil and 1% cholesterol powder) for first four weeks in order to induce hyperlipidemia. After first four weeks, 2% date seed powder of three Pakistani varieties namely Dhakki, Khudrawi and Desi was added to the diet of three experimental hyperlipidemic groups for the next four weeks. Body weight and blood samples were taken at zero, 4th and 8th week of study. Serum was analyzed for total cholesterol, LDL-cholesterol, HDL-cholesterol and serum triglycerides. LDL/HDL ratio and AIP were calculated. Results: It was observed that date seed powder of the three varieties significantly decreased total cholesterol, serum triglycerides and AIP. There was no significant change in body weight, HDL-cholesterol, and LDL/HDL ratio. LDL cholesterol was decreased significantly only by Khudrawi date seed powder Conclusion: It was concluded that date seed powder has marked anti hyperlipidemic properties. However, the difference in appearance, taste and price of different dates does not affect their lipid lowering capacity.

Keywords: Hyperlipidemia; Date-seed, P.Dactylifera; Lipid profile

INTRODUCTION

Hyperlipidemia is defined as increase in one, multiple or all types of lipids in the blood stream. It can be due to a defect in the enzyme system or secondary to any metabolic disease. Hyperlipidemias are classified according to their etiology. Hyperlipidemias caused as a result of defects in single gene or environmental factors are labeled as primary hyperlipidemias. However, if the deranged lipid profile occurs due to a metabolic disease like diabetes, alcoholism, thyroid disorders, cirrhosis of bile duct or liver, it is termed as secondary hyperlipidemia. Hyperlipidemia is one of the major risk factors for coronary vascular disorders and other atherosclerotic diseases, which are the leading cause of deaths all over the world. In 2012, approximately 111,000 deaths in Pakistan were caused by atherosclerosis , accounting for 19% of total deaths per annum. In addition to medical treatment, lifestyle modification is also very important in order to prevent and treat hyperlipidemias. Medicinal plants have been used in various traditional systems, as they have immense potential against numerous diseases. Some of these plants have also shown their beneficial effect in hyperlipidemia. In comparison to medical management, patient compliance is better in nutraceutical management as there is a lesser risk of adverse effects, allergies and contraindication.

Researchers have demonstrated the role of ginger juice, flaxseed and date palm leaf extract in the management of hyperlipidemia. Date palm (Phoenix Dactylifera) belongs to the Arecaceae family. It has a monocotyledonous perennial fruit and is a major crop in the Middle East. It is among a few plants that can survive in the tough arid environment and is highly regarded for the nutritional value that the palm tree fruit provides.

About 150 different types of Phoenix dactylefera are grown in Pakistan, which makes it one of the largest cultivars of dates all over the world. More than half of the total dates produced in Pakistan are grown in Sindh, where district Khairpur is the main cultivar, producing 85% of the dates in the region. Sukkur, Larhkana and Ghotki are also among the major date producing regions of Sindh. In Punjab, Multan, Dera Ghazi Khan and Muzaffargarh are the main date producing regions due to favorable climate. Studies have been conducted to observe the benefits of the date palm, either from its fruit or seed, and it has been found that date seed extract possesses several highly beneficial properties such as hepatoprotective and anti hyperglycemic. One study has shown
improvement in early diabetic complications in experimental animals.14

In the past, date seeds were considered as wastage, but in the recent years various parts of *P. dactylifera* including seeds, roots and gum from the tree are being used in the treatment of different diseases including sore throat, cold, toothache, diarrhea, gonorrhea, and urinary tract infections.15 This provides opportunity to the researchers to analyze the therapeutic potential of all the parts of this plant.

Among all the date seed varieties, Ajwa date is the most famous and beneficial variety and a recent study has demonstrated antihyperlipidemic effect of its seeds.16 As Ajwa date is expensive and not affordable by majority of population, this experimental study was designed to find the beneficial effects of Pakistani date seeds on lipid profile and to see if the difference among cost, taste and quality of different varieties exist in their nutraceutical role.

**MATERIAL AND METHODS**

Thirty male albino rabbits weighing 1–1.5 kg were purchased from local market and kept for acclimatization for two weeks in the animal house of PGMI. The rabbits were divided into five groups, having six animals in each group. One group (Group A: normal control) was given normal rabbit chow throughout the study period of eight weeks. The remaining four groups were fed high fat diet containing 1% cholesterol and 4% coconut oil for first four weeks in order to induce hyperlipidemia.17 After first four weeks, 2% date seed powder18 of three Pakistani varieties namely Dhakki, Khudrawi and Desi was added to the diet of three experimental hyperlipidemic groups (Group C, D and E) for the next four weeks, while high fat diet (HFD) control group (Group B) continued to take high fat diet alone.

Three different dates varieties of Pakistan including Dhakki, Khudrawi, and Desi were purchased from the date farms of district Muzaffargarh. Their seeds were separated from the flesh, washed and then dried in the air. The dried seeds were finely ground to powder in a heavy duty grinder and were stored in a cool dry place.19 About 400 grams of date-seed powder of each variety was used during the four weeks of the study period. Body weights and blood samples were taken at the start of study, at the end of 4th week and at the end of 8th week of study. Serum was analyzed for total cholesterol, LDL-cholesterol, HDL-cholesterol and serum triglycerides. Serum lipid profile estimation was done by enzymatic end point method, using commercially available kits (Analyticon) with standardized spectrophotometer(Pictus B). LDL/HDL ratio and AIP were calculated. Atherogenic index of plasma was calculated using a standard formula, log(triglyceride/HDL). Data were transcribed into computerized package, i.e., GraphPad Prism 5.0. After checking normality by Shapiro Wilk test, data was presented as Mean±SD. Analysis of Variance (ANOVA) followed by post hoc Tukey’s test was applied to test the significance of difference in study groups. Paired sample two tailed t-test was used to see difference between times within a group.

**RESULTS**

It was observed that date seed powder of the three varieties significantly decreased total cholesterol, serum triglycerides and AIP. There was no significant change in body weight, HDL-cholesterol, and LDL/HDL ratio. LDL cholesterol was decreased significantly only by Khudrawi date seed powder.

In group A, the mean body weight increased from 1.17±0.15 kg at zero week to 1.21±0.10 kg at 8th week. In group B, it increased from 1.21±0.15 at zero week to 1.33±0.15 kg at 8th week. Among the three experimental groups, C, D and E, the mean body weight increased from 1.21±0.16, 1.21±0.10 and 1.18±0.06 kg at zero week to 1.32±0.11, 1.34±0.07 and 1.35±0.04 kg at 8th week respectively. This change in mean body weight was statistically insignificant.

Serum total cholesterol increased from 0 to 4th week significantly in all the groups except normal control group. From 4th to 8th week, all the three date seed varieties significantly decreased serum total cholesterol however it was still higher than the baseline. The maximum decrease was observed with Khudrawi date seed powder (Figure-1). At 8th week, when compared with the HFD control group, the total cholesterol level in the three experimental groups was significantly lower with a p-value of 0.01, 0.003 and 0.003 respectively.

Serum triglyceride levels increased significantly from zero to 4th week in all the groups except normal control group. From 4th to 8th week, the three date seed varieties significantly decreased serum triglyceride almost upto baseline (Figure-2). At the end of study period, serum triglyceride values of the three experimental groups C, D, E were significantly lower than the HFD control group, i.e., group B, with p-value <0.001 in each case. LDL cholesterol increased significantly from zero to 4th week in all the groups except normal control group. From 4th to 8th week, LDL cholesterol level decreased with all the three date seed varieties (Figure-3). However, this decrease was statistically significant with only Khudrawi date seed powder with p-value of 0.01. Difference among the three experimental groups was insignicant. At 8th week, LDL cholesterol value of group D was significantly lower than the HFD control group i.e group B, with p-value 0.01, however in case of group C and E, this difference was insignificant. When each group was analyzed over time using paired t test, the difference in the serum HDL cholesterol in all the groups at any two
times, was found out to be statistically insignificant, except for the natural increase in the HDL level of normal control group between zero and 8th week (Figure-4). Since no statistically significant difference was found by ANOVA, post hoc test was not applied. A significant increase was observed in all the groups from zero to 4th week except normal control group. From 4th to 8th week there was a significant decrease in the means of AIP of group C and D. In case of group E, though considered statistically insignificant, however the decrease in AIP was not ignorable having p-value 0.05 (Figure-5). At 8th week, the levels of the three experimental groups were significantly lower than the HFD control group, i.e., group B, with p-value 0.000 in each case. LDL/HDL ratio was calculated at each sampling, and it was observed that the levels increased from zero to 4th week with p values 0.002 in group C, 0.001 in group D and 0.02 in group E. There was no significant decrease in the means of these three groups from 4th to 8th week. At 8th week, LDL/HDL ratio of group C, D and E, when compared to the HFD control group, i.e., group B, was not significantly different, however in case of group D, it cannot be ignored as the p-value is 0.053.

DISCUSSION

This study was devised to evaluate the antihyperlipidemic action of date-seed powder of three different Pakistani dates in diet induced hyperlipidemia in rabbits.

Rabbits were selected as the experimental animal because lipid metabolism mechanics are similar to humans and they are very sensitive to
dietary cholesterol resulting in easy induction of hyperlipidemia. Although studies have been performed on either sex, but male rabbits are preferred in case of hyperlipidemia because female sex hormones are protective and thus interfere with induction of hyperlipidemia.

It was observed that all the three date seed varieties significantly reduced serum total cholesterol, serum triglyceride and atherogenic index of plasma. The decrease in LDL cholesterol was statistically significant only for Khudrawi date-seed powder. The change in HDL cholesterol and body weight was statistically insignificant.

To the best of our knowledge, no study was available on date seed powder of any variety except Ajwa date seed powder. Ajwa date-seed powder showed a significant decrease in serum total cholesterol with a p-value of <0.001. In the present study, serum total cholesterol decreased in the experimental groups with a p-value of 0.01(387±99mg/dl) in group C, 0.003(303±88mg/dl) in group D & 0.003(318±121mg/dl) in group E. The slight difference in the results of ajwa dateseed powder and the date seed powders used in the present study maybe due to difference in their phytochemical composition; as concentration of constituents tend to vary with respect to plant origin, atmosphere, soil and cultivar land. Date-seed powders of the three experimental varieties, included in the present study, were found to decrease serum triglyceride with a p-value of <0.001. The decrease in triglyceride level by date-seed powder in the present study is comparable to effect of Ajwa date-seed. The LDL cholesterol was significantly decreased in the Khudrawi group (p-value 0.01), whereas the decrease by Dhakki and Desi date-seed powder was not statistically significant. Previous studies have proven polyphenols, flavanoids and fibers to possess antioxidant and thus, lipid lowering action. However, there amounts are variable in different plants and their parts. When these results were compared with Ajwa date seed powder, it was observed that Ajwa date seed has a stronger LDL lowering effect than the present study with p-value <0.001.

The HDL cholesterol did not increase significantly in any of the experimental groups in the present study (data not shown), whereas Ajwa date seed increased HDL cholesterol with a p-value of <0.001. This shows that Ajwa date seed powder is superior to date seed powder of the present study in cardioprotective action. Comparison of phytochemical properties of Ajwa dates with various local dates has proven its supremacy in antioxidant concentrations and therapeutic potential.

Atherogenic index of plasma (AIP) was calculated using data of triglycerides and HDL, and it was found that all the three experimental date-seeds lowered AIP with a p-value of <0.001, while Ajwa date-seed powder reduced it with a p-value 0.01. A marked reduction in AIP by date-seed powders under study, predict their protective potential in cardiovascular diseases, because AIP is considered to be a major predictor of diseases like hypertension, diabetes and other cardiovascular complications.

Date seed powders of different varieties all over the world have been studied for their phytochemical composition. It has been found that date seeds have polyphenols, flavanoids, tannins and alkaloid. The antioxidant role of polyphenols and flavanoids in flax seeds and grapes seed has been demonstrated in the past. Hence, it can be proposed that the antihyperlipidemic action of date seed powder may be due to the antioxidant and radical scavenging activity of agents like polyphenols and flavanoids present in it. The present study showed similar results of all the three date seed varieties without any significant difference among each other except for the effect on LDL cholesterol levels. It may be due to the fact that different constituents in the date-seed powder are responsible for different actions. Also, each parameter of the lipid profile has its own mechanism of control and management. A study conducted on eighteen different date seeds of UAE showed a significant difference in amount of polyphenols and flavanoids, however dietary fiber was comparable in all the varieties.

Assuming a similar scenario in the present study, it can be proposed that the effect on total cholesterol and triglycerides may involve more of the dietary fibre in the date-seed, and act at the level of absorption from GI tract. LDL cholesterol may have a more complex mechanism, involving the action of polyphenols on the cellular level. The fact that no effect was seen on the HDL levels, with any of the dates, further strengthens this belief. However, further research is required to confirm these propositions.

CONCLUSION
From the result of this study, it can be concluded that Pakistani date-seed varieties of different prices have similar beneficial effects on serum total cholesterol, serum triglycerides and atherogenic index of plasma. These dates may be used as a cheap nutraceutical source for the treatment of hyperlipidemia.

AUTHORS' CONTRIBUTION
MM: Literature search, concept, study design, data collection, analysis, interpretation, write-up. SZ:
Literature search, data analysis, manuscript writing. MF: Manuscript writing, proof reading. SC: Supervision of research, proof reading. SM: Literature search, data collection. AMR: Write-up, proof reading.

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