

Influence of Varying DatePalm (*PhoenixDactyliferaL.*) Powder Levels In Hyperlipidemic Rat Model

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ABSTRACT

Hyperlipidemia is a new global burden. Sun dried date flesh is an innovative way to store them. It is necessary to analyze lipid lowering potential of date palm powder. To find out the therapeutic effect of Date palms (*Phoenix dactylifera L.*) powder with different concentrations on blood lipid profile among hyperlipidemic experimental rats. Total 30 rats of 200±20g were divided into 6 treatment groups. 1st was positive control (T0) group, 2nd was negative control (N0) and 3rd, 4th, 5th, and 6th (T1, T2, T3, T4) were treatment group. All rats were exposed with hyperlipidemia except group N0. Blood lipid levels (TC, TG, LDL and HDL) were analyzed at zero day and 28th day of the treatment. Group T1 to T4 were fed on 3ml, 5ml, 8ml and 10ml of date powder solution for 28 days. After the consumption of date powder, rat's weight reduced from 259.40±10.06 to 247.20±9.65, TC level declined from 150.00±6.63 to 145.00±7.48, LDL reduced from 120.80±4.96 to 111.80±5.80, TG reduction seen from 159.20±7.25 to 150.00±7.84. While HDL was increased from 40.00±4.12 to 45.00±4.00 with all the result statistically significant ($p \leq 0.05$). The results of proximate analysis of date powder contains 1.94±0.82% ash content, 0.53±0.46% fat content, 5.23±0.43% fiber, 2.43±0.47% protein and 85.80±5.64 NFE. TPC 253.11±28.19, TFC 43.18±3.43 and 15.16±1.60 DPPH. Date palm powder reduced hyperlipidemia significantly. Raised HDL level (p -value 0.038), reduce TC, LDL and TG levels.

INTRODUCTION

Elevated levels of total cholesterol level, triglycerides and low-density lipoprotein cholesterol in blood serum are referred to as hyperlipidemia. [1]. Globally, cardiovascular illnesses are the leading cause of deaths. 60% of the world's CVD burden is located in south Asia. Pakistan, which has a high mortality rate from coronary artery disease, comes in at number 17 among them. The latest WHO data, released in April 2011, show that 196,258 fatalities in Pakistan were caused by CHD (15.36%). The main risk factor for coronary heart disease is hyperlipidemia. Almost fifty-six percent of patients with hyperlipidemia have CHD at some point in their lives, particularly between the ages of 40 to 60 [2]. Around nineteen percent of all annual deaths in Pakistan, or approximately 111,000 deaths, are due to atherosclerosis [3].

Every year, almost Twelve million people die from cardiovascular disease worldwide. High levels of cholesterol, especially LDL cholesterol in diet in combination with in the form of saturated fats and cholesterol with the combination of age, genetic history, high blood pressure, and lifestyle choices, are very important factors that affect the development of cardiovascular events [4]. By 2030, it is predicted that nearly 24 million individuals will encounter cardiovascular issues [5].

The much more prevalent type of dyslipidemia is hyperlipidemia (which includes any hypo and hyper lipid levels). A spike in blood lipid levels distinguishes the group of diverse illnesses known as hyperlipidemia. TG, phosphatide, cholesterol esters as well as cholesterol are some of these lipids. Dietary fat being absorbed by the small intestine and converted as chylomicrons, that are later transported to peripheral organs by the circulation. Chylomicrons are broken down by lipase enzyme (LPL), and fatty acids are then transported to subcutaneous and visceral tissues and muscle. Chylomicron fragments are taken up by the liver, which then begins the production of VLDLs. The liver is where High Density Lipoprotein, or HDL, is made. The liver receives cholesterol through HDL as it leaves the body. This is why HDL is referred to as the good cholesterol. High density lipoprotein has antioxidant properties as well as work as heart friendly component. Fundamentally, hyperlipidemia can be categorized as primary (familial) or secondary (acquired), each of which is brought on by a different underlying condition. Primary hyperlipidemia is characterized by certain genetic abnormalities. Idiopathic hyperlipidemia occurs occasionally and has no apparent aetiology [6].

One of the most popular plant groups in cultivation is the date palm (*Phoenix dactylifera* L.). They have been crucial to human diet even from ancient times. The pits and flesh of a date palm fruit are both edible. The pulp is an abundant source of dietary fibre, carbs, as well as some vital minerals and vitamins [7].

NUTRITIVE VALUE OF DRIED DATES PALMS

According to reports, date fruit is a good source of food with a high amount of nutrients. It is abundant in minerals, B complex vitamins including B1 (thiamine), B2 (riboflavin), B3 (niacin), pantothenic, pyridoxine, and folic acid as well as carbs, protein and dietary fibre. Additionally, it is thought to be a good source of Vit A, E and C which have antioxidative properties. The flavonoid composition of date palm fruit is made up of rutin, anthocyanins, isoquercetrin, apigenin, quercetin, quercetin, procyanidins and luteolin [8]. A good source of minerals like calcium, potassium, zinc, copper and folate is date powder. The findings indicated that date fruit contained adequate levels of minerals as well as phosphorus both before and after

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drying. This occurs as a result of the components' higher content after drying due to the concentration impact [9].

Alqarni et, al 2019 conducted an experiment to find lipid lowering potential and antioxidant activity of date palm (Ajwa) polyphenolic extract (DPE). hyperlipidemic rats' body weights and hepatic weight, total hepatic lipid, Low density lipoprotein, very low-density lipoprotein cholesterol and tri-glyceride levels were all significantly decreased after receiving oral doses of 25 mg DPE/kg, 50 mg DPE/kg, and 100 mg date polyphenol extract per kilogram of body weight. Besides that, date polyphenol extract therapy increased ($p \leq 0.05$) the High-density lipoprotein content and activity of antioxidant enzymes in a dosage manner, stabilizing plasma lipids and boosting the body's natural defenses against free radicals [10].

Khalid et, al in 2020 conducted a study on the fruit of the date palms, in which he founds it as a potential and important source of nutrients, including carbohydrates, proteins, dietary fibers, minerals, and vitamins, as well as proanthocyanins, quercetin, rutin and apigenin. Date palm's phytochemical content results in a considerable decrease in LDL cholesterol and very VLDL-C, as well as an increase in HDL-C. Additionally, they raise the levels of serum enzymatic antioxidants that prevent the generation of free radicals. . According to the present studies, *Phoenix dactylifera* antioxidants can prevent coronary irregularities by regulating plasma lipids level, decreasing oxidative stress, sustaining Na metabolism, and enhancing contractions of heart muscle contraction [11].

Arshad et al in 2018 proposed research to view the lipid lowering effect of ajwa dates, aseel, Hallawi and Khudravi. 6 rats groups were included in study. Ajwa has high amount of reducing sugar. Aseel have high level of phenolic content as compare to others. Ajwa date extract lower cholesterol (110 mg/dl) and Hallawi extract by 103mg/dl. These both dates showed same results in lowering low density lipoprotein, TC, TG and have nephroprotective potential. Among all types Hallawi dates extract can be consider as beneficial as lipid lowering agent. . Proximate analysis shows that ajwa and hallawi have less crude fiber, ajwa have lowest fat content. All of the varieties have low sodium level but higher in potassium [12].

Pakistan stood on 17th number in the prevalence of hyperlipidaemia. Date palm fruits (*Phoenix dactylifera* L.) are one of the most cultivated fruits among Arab countries. Pakistan is the 7th largest exporter of dates palms. High flavonoid and phenolic content makes date fruit as best fruit to reduce oxidative stress. High fiber and low-fat content make them cardioprotective fruit. It has proven low sodium content in dates palms. To find out the therapeutic effect of Date palms powder (*Phoenix dactylifera* L.) with different concentrations on blood lipid profile was the main objective of this study.

METHODOLOGY

STUDY DESIGN

Experimental study design (Rat bioassay). STUDY

DURATION

9 months after approval of synopsis. SAMPLING

TECHNIQUE

Simple Random Sampling SAMPLE

SIZE

Total number of rats were 30 with 5 rats in each group.

PROCUREMENT OF MATERIALS

Dried dates were purchased from local market of Lahore.

PREPARATION OF DATE POWDER SUPPLEMENTATION

After removing dirt particles the dried dates were then washed, pits were removed, and sun dried for three days and grounded into fine powder for further analysis.

PROXIMATE ANALYSIS

Date palm powder was analyzed to find out its chemical composition. Moisture, Ash, Fat, Fiber, and Protein, NFE as well as phenolic content, flavonoid content and antioxidant activity (DPPH) were analyzed using different equipment.

MOISTURE-FREE DATE PALM SAMPLE EXPERIMENTAL DESIGN

Animals gathered after observing inclusion criteria were divided randomly into 6



groups by placing 5 rats in each treatment group. Date powder solution was given to rats by using gavage for four weeks. At the end of the study, rats were kept on fasting overnight and then anesthetized by injecting Ketamine. Blood samples were collected by cardiac puncture method and blood was analyzed to determine lipid profile values of each rat of every group. Body weight of experimental rats were measured at the starting of the treatment or at the day zero and at the end of the treatment or at 28th day by using weight balance.



MARKED EACH RAT SEPARATELY

ESTIMATION OF BLOOD LIPID LEVEL

Blood sample were collected by cardiac puncture of experimental rats at the starting of treatment or at day zero and at the last day of treatment or at 28th day. Four lipid profile parameters were included in the study that were Total cholesterol (TC), Triglycerides (TG), LDL and HDL levels.



DRAWING BLOOD FOR LIPID PROFILE

TREATMENT PLAN

GROUPS	TREATMENT
T ₀	First group of rats was fed on 40g of animal fat with 60g of normal diet and considered as positive control group
N ₀	Second group of rats was fed with 40g of normal diet throughout the experiment and remained as “Controlled Group” without the provision of date palm powder and without cholesterol in normal diet
T ₁	date powder solution in distilled water per day.
T ₂	Forth group of hyperlipidemic rats (each) was fed with 5ml of date powder solution in distilled water per day.
T ₃	Fifth group of hyperlipidemic rats (each) was fed with 8ml of date powder solution in distilled water per day.
T ₄	Sixth group of hyperlipidemic rats (each) was fed with 10ml of date powder solution in distilled water per day

STATISTICAL ANALYSIS PROCEDURE

Data was entered and subjected to statistical analysis using SPSS version 25. Mean $\pm$ standard deviation was used for quantitative variables. Frequency and percentages were used for qualitative variables. ANOVA was used to analyze mean differences and the level of significance of Six groups. The level of significance was taken as ($p \leq 0.05$).

RESULTS**TABLE 1: PROXIMATE ANALYSIS OF DATE PALM POWDER**

Proximate analysis	Mean $\pm$ SD
Moisture	7.00 $\pm$ 0.25
Ash	1.94 $\pm$ 0.82

Fat	0.53±0.46
Fiber	5.23±0.43
Protein	2.43±0.47
NFE	85.80±5.64
Total phenolic content (GAE/g)	253.11±28.19
Total flavonoid content (QE/g)	43.18±3.43
DPPH (µg/mL)	15.16±1.60

Proximate analysis of date palm powder showed 7.00±0.25% of moisture content, 1.94±0.82% ash content, 0.53±0.46% fat content, 5.23±0.43% fiber, 2.43±0.47% protein and 85.80±5.64 nitrogen free extract. Date palm powder contains total phenolic content of 253.11±28.19, total flavonoid content of 43.18±3.43 and 15.16±1.60 antioxidant activity (DPPH).

TABLE 2: AVERAGE WEIGHT CHANGES BETWEEN DAY 0 AND DAY 28 (PRE AND POST TREATMENT)

Sr no	Parameters (g)	Mean±SD	P-Value
1	T0 Weight Day 0	332.80±5.63	0.030
2	T0 Weight Day 28	350.60±7.95	
3	N0 Weight Day 0	272.00±11.20	0.009
4	N0 Weight Day 28	271.80±10.56	
5	T1 Weight Day 0	257.80±10.30	0.05
6	T1 Weight Day 28	253.60±9.52	
7	T2 Weight Day 0	260.60±4.97	0.248
8	T2 Weight Day 28	258.60±5.77	
9	T3 Weight Day 0	264.00±5.24	0.19
10	T3 Weight Day 28	258.40±8.44	
11	T4 Weight Day 0	259.40±10.06	0.001
12	T4 Weight Day 28	247.20±9.65	

This experimental study held among rats. Weight was measured at pre and post treatment. All groups were fed upon different amount of date palm powder solution. Above table no.2 shows mean ± SD values regarding weight of all groups measured at day zero and at the 28th day of treatment. Weight of all treatment groups are statistically significant except group T2 (P=0.248) and T3 (P=0.19).

TABLE 3: COMPARISON OF WEIGHT AMONG ALL GROUPS AT DAY 0 (PRE- TREATMENT)

	Groups	Mean±SD	P value
Weight (g)	T0	332.80±5.63	0.000
	N0	272.00±11.20	
Day 0	T1	257.80±10.30	
	T2	260.60±4.97	
	T3	264.00±5.24	

Table 3 represents Mean ± SD values of weight measurement among rats included in all groups at zero day or prior to treatment. Results of weight at day 0 were statically significant as P=0.000

TABLE 4: COMPARISON OF WEIGHT AMONG ALL GROUPS AT 28TH DAY (POST TREATMENT)

	Groups	Mean±SD	P value
Weight (g)	T0	350.60±7.95	0.000
	T4	259.40±10.06	
Day 28	N0	271.80±10.56	
	T1	253.60±9.52	
	T2	258.60±5.77	
	T3	258.40±8.44	
	T4	247.20±9.65	

Above, table no.4 represents Mean ± SD values of weight measurement among rats included in all groups at 28th day of treatment or at post treatment. Results of weight at 28th day were statically significant as P=0.000

TABLE 5: COMPARISON OF TOTAL CHOLESTEROL LEVEL AMONG ALL GROUPS AT 0 DAY (PRE-TREATMENT)

	Groups	Mean±SD	P value
TC (mg/dL)	T0	188.60±7.36	0.000
	N0	131.60±2.70	
Day 0	T1	149.60±9.65	
	T2	150.80±4.49	
	T3	152.60±5.50	
	T4	150.00±6.63	

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Above table represents Mean $\pm$ SD values of total cholesterol (TC) level at day zero among all treatment groups. Results were statically significant as $P=0.000$. The results were obtained by using ANOVA.

TABLE 6: COMPARISON OF TOTAL CHOLESTEROL LEVEL AMONG ALL GROUPS AT 28 DAY (POST-TREATMENT)

	Groups	Mean $\pm$ SD	P value
TC (mg/dL)	T0	211.00 $\pm$ 14.07	0.000
	N0	129.20 $\pm$ 3.42	
Day 28	T1	144.80 $\pm$ 10.63	
	T2	150.00 $\pm$ 6.70	
	T3	147.60 $\pm$ 5.02	
	T4	145.00 $\pm$ 7.48	

Above table 6, represents Mean $\pm$ SD values of total cholesterol (TC) level at day 28 among all treatment groups. Results were statically significant as $P=0.000$. The results were obtained by using ANOVA.

TABLE 7: COMPARISON OF LDL LEVEL AMONG ALL GROUPS AT 0 DAY (PRE- TREATMENT)

	Groups	Mean $\pm$ SD	P value
LDL (mg/dL)	T0	149.40 $\pm$ 13.46	0.000
	N0	111.20 $\pm$ 6.41	
Day 0	T1	126.80 $\pm$ 6.18	
	T2	121.40 $\pm$ 7.02	
	T3	122.60 $\pm$ 3.64	
	T4	120.80 $\pm$ 4.96	

Table number 7 shows Mean $\pm$ SD values for low density lipoprotein (LDL) level among rats included in all treatment groups including T0, N0, T1, T2, T3, and T4. Results were statistically significant as $P=0.000$.

TABLE 8: COMPARISON OF LDL LEVEL AMONG ALL GROUPS AT 28 DAY (POST-TREATMENT)

	Groups	Mean±SD	P value
LDL (mg/dL) Day 28	T0	163.60±13.14	0.000
	N0	113.80±9.01	
	T1	122.40±5.94	
	T2	119.60±5.02	
	T3	115.00±4.79	
	T4	111.80±5.80	

Table no. 8 shows Mean ± SD values of LDL level among rats included in all groups at 28th day of treatment or at post treatment. Results of weight at 28th day were statically significant as P=0.000

TABLE 9: COMPARISON OF TRIGLYCERIDE LEVEL AMONG ALL GROUPS AT 0 DAY (PRE-TREATMENT)

	Groups	Mean±SD	P value
TG (mg/dL) Day 28	T0	167.60±10.18	0.001
	N0	138.40±7.30	
	T1	151.80±8.78	
	T2	153.40±9.37	
	T3	150.00±6.44	
	T4	150.00±7.84	

Table 9, mentioned above represents comparison of triglyceride level (TG) Mean±SD values among rats included in all treatment group at zero day. While the results showed statically significant as P= 0.017

TABLE 10: COMPARISON OF TRIGLYCERIDE LEVEL AMONG ALL GROUPS AT 28 DAY (POST-TREATMENT)

	Groups	Mean±SD	P value
	T0	152.40±7.02	0.017
	N0	141.40±4.92	

Day 0	T1	156.60±9.44	TG (mg/dL)
	T2	156.80±10.32	
	T3	155.60±5.54	
	T4	159.20±7.25	

The table number 10 shows Mean±SD values of triglyceride (TG) level among rats included in all groups at 28th day of treatment or at post treatment. Results of weight at 28th day were statically significant as P=0.001.

TABLE 11: COMPARISON OF HDL LEVEL AMONG ALL GROUPS AT 0 DAY (PRE- TREATMENT)

	Groups	Mean±SD	P value
HDL (mg/dL) Day 0	T0	46.80±3.70	0.074
	N0	40.20±4.96	
	T1	46.20±3.70	
	T2	40.40±6.84	
	T3	41.00±3.74	
	T4	40.00±4.12	

Above table represents Mean±SD values of HDL level (high density lipoprotein) among rats included in all groups at 0 day of treatment or at post treatment. Results of weight at 0 day were statically not significant as P=0.074

TABLE 12: COMPARISON OF HDL LEVEL AMONG ALL GROUPS AT 28 DAY (POST-TREATMENT)

	Groups	Mean±SD	P value
HDL (mg/dL) Day 28	T0	38.80±2.94	0.038
	N0	39.80±7.29	
	T1	48.60±4.03	
	T2	41.80±6.64	
	T3	46.00±4.00	
	T4	45.00±4.00	

The table number 12 shows Mean±SD values of HDL level (high density lipoprotein) among rats included in all groups at 28th day of treatment. Results of weight at 28th day were statically significant as P=0.038.

DISCUSSION

The purpose behind choosing the topic of date palm fruit powder effect on heart and lipid profile is solely based on emerging issues of hyperlipidemia and its prolonged effect on heart health. Pakistan is one of those countries that are getting towards higher prevalence of hyperlipidemia and coronary heart diseases especially in younger ages. As Pakistan falls in developing country, less affordability of hyperlipidemic medications and their long term side effects proceeds towards the need of research on natural food products such as date palm to overcome the chances of CVD without any adverse effects. As per the finding by WHO around 2.6 millions of people died due to hyperlipemia every year. Hyperlipemia is 40% prevalent in males while 37% in females according to world health organization [13]. In a study conducted by Abiola et al, providing date extract to hypercholesteremic rats showed reduction in lipid profile level. Total cholesterol was declined to $85.27 \pm 3.74\%$, HDL was increased by $44.61 \pm 0.67\%$, LDL and triglycerides were reduced as 30.78 ± 1.35 and $100.6 \pm 1.83\%$ respectively. These findings were similar to this study with minor moderation [14].

CONCLUSION

After carefully and keen analysis of the results of this study it can be concluded that date palms (*Phoenix dactylifera L.*) powder have positive effect in the reduction of hyperlipidemia among experimental rats. Date palm powder consist of several beneficial component such as proteins in higher level as compare to any other fruit. Dates have great antioxidant levels like high content of flavonoid and good amount of phenolic content as well as higher antioxidant scavenging activity (DPPH). High fiber and very low amount of fat in dates makes them one the ideal fruit to reduce elevated lipid profile in blood serum hence protect the body from several serious health conditions before their extension into advanced form. Date palm is one of the few fruits that have low sodium and high potassium levels, that can makes them to normalize blood pressure in body. This study shows that date palm powder have positive effect to lower elevated lipid profile levels due to the presence of beneficial components in them. The present study shows declined in triglycerides level, total cholesterol, low density lipoprotein, while raised high-density lipoprotein level in blood of hyperlipidemic rats.

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