Evaluation of therapeutic potential and anti-hyperchlostrolemic effects of prunes in albino rats: An experimental study

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Abstract: Hyperlipidemia is a global epidemic that causes various cardiovascular diseases (CVDs). Prunes include fiber and numerous phenolic compounds that decrease cholesterol by decreasing LDL oxidation and supporting heart health. This study examined the therapeutic effects of *Prunus domestica* prunes on plasma fatty acids in albino rats after ingesting prune pulp. After chemical examination, prunes were proximately examined for nutritional content. *Prunus domestica* pulp was given to hyperlipidemic rats for two months in a clinical trial. 12 albino rates and divide into 3 groups. First group was controlled, others experimental. The study's 15th, 30th, and 60th days evaluated lipid profile. The following study was analyzed using 2 way anova. Prunes have enough fiber, minerals, and polyphenols to affect hyperlipidemic rats. GIII rats lower LDL, weight, and HDL more than GI and GII.

Keywords: Low density lipids, weight loss, hyperlipidemia.

INTRODUCTION

Modern technology and processed meals are appealing due of their stimulating characteristics. Functional and effective foods improve consumer health and reduce the risk of illness (Shahidi, 2009). Eating enough prunes and prune-based products can lower plasma triglycerides and LDL cholesterol and raise HDL cholesterol (Yousse, 2007). Different quality Rosaceae fruits may be clustered or uncrowded and have buds on their body parts. 20–41 plum varieties. Japanese and European plums are more valuable worldwide (Tewfik & Tewfik, 2008).

Experimental study indicates that moderate taking of natural items as compared to processed like veggies etc. May reduce the chances of heart diseases, due to the existence of phenolic compounds (flavonoids; polyphenols), which ascribe for its antioxidant and antiinflammatory properties. Veggies or fruits perhaps beneficial in overcome the death rate in CVDs patients by lowering the oxidation of cholesterol and foods that are rich in soluble fiber have a hypocholesterolemia effect (Slavin and Lloyd, 2012).

Generally, dehydration of plum makes prune. Many

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products of prunes like jam, puree, pulp and juices are usually processed in the market. Nutritional composition in prunes is 30.89% water, 65% carbohydrates, including 7.5% dietary fiber, 2.2% protein and less than 1.1% fat. Prunes are richest origin of K vitamin and moderate amount of certain B vitamins and edible minerals. Phytochemicals including phenolic compound (mainly chlorogenic acid, neochlorogenic acids and oligomeric proanthocyanin) and sorbitol are present in prunes (Stacewicz-Sapuntzakis *et al.*, 2001).

The World Health Organization recommends that we eat 7 to 8 portions of fruit and vegetables every day. Several studies demonstrated that prune is richest source of fiber both soluble and insoluble fibers, about 60 percent of the cholesterol lowering dietary fiber in dried plums is pectin. Viscous fiber regulating the cholesterol level in the body by stiffen the contents of intestinal tract and may impaired the absorption of sugar, decrease sugar response after eating and decrease lipid absorption, that helps in regulating the cholesterol level in the body. It also contains simple sugars mainly xylo-oligosaccharides moreover micronutrients as minerals or vitamins (Stacewicz-Sapuntzakis *et al.*, 2001; Putnam *et al.*, 2007).

MATERIALS AND METHODS

Preparation of sample

The prunes were purchased from local market and identified by Nutritionist Dr. Anam from the University of Agriculture, Faisalabad. The prunes were cleaned and free from dust particles and other impurities and also clear of damage and wrapped in polythene bags to avoid any contamination. Prunes were soaked into water over night and then mash it with hands and make pulp and add in the diet of albino rats dosage of prunes pulp was 12gm and 24gm daily for 16-43 days.

Collection of rats and Induction of hyperlipidemia

The male albino rats were purchased from the institute of public health along with weight of 10g and they kept in The rats will be acclimatized by feeding on basal diet for a period of one week. The environmental conditions will be control throughout the trial like temperature $(23\pm2^{\circ}C)$ and relative humidity $(55\pm5\%)$ along with 12 hours light-dark period. The high cholesterol diet consisting of animal fat was provided to the normal rats for taking variation in their lipid profile i.e. cholesterol, good cholesterol (high density lipoprotein HDL), bad cholesterol (low density lipoprotein LDL) and triglycerides. The variance cholesterol values were given in table 3.

Experimental modeling and rats-grouping

The investigation was conducted on twelve male mature rats.Animal were divided arbitrary into tow alike groups. All rats were accommodating to free access to normal diet and water for 7days before initiating the research for adjustment. After adaptation period, the rats were divided into the subsequent groups;

Study I: Hypercholesterolemic rats with normal diet

In study I, high cholesterol diet consisting animal fat was provided to the normal rats to take discrepancy in their lipid profile i.e. Cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides. Then, normal diets were provided to respective rat group.

Study II: Hypercholesterolemic rats with prunes diet

In inquiry II animal fat diet was provided to normal rats to change their lipid profile. Then prunes pulp containing diet were fed to appropriate group at the equivalent time to integrate their influence on relevant category.

Treatment plan for efficacy study

The study was conducted on albino rats. Twelve hyperlipidemic subjects were randomly designate and distributed into three groups. Group I: Control group (n=4) and Group II and GIII: Experimental groups (n=8). Where n= number of rats. Control group was expressed as GI. Experimental groups GII & GIII were given 12g and 24g of prunes respectively. The pulp of prunes was added in their regular diets. The study was conducted for 60 days. Initially blood sample was taken from each rat and

Reduction in body weight

Weight of the rats determine by the weight machine at 0, 30^{th} and 60^{th} day. Reduction in body weight of rats was noticed from both experimental groups usually throughout the study period, whereas, no difference measured in control group. The values of variance of weight were given in table 5.

Proximate analysis

The prunes were subjected to proximate analysis to determine the moisture content, crude protein, crude-fat, crude-fiber, ash and minerals following to their appropriate methods (AOAC, 2006).

Biochemical analysis

To determine the level of different parameters, overnight fastened rats in each group were anaesthetized and blood taken from cardiac puncture, and were collected in tube. Blood samples was drawn from each rat at 0, 30 and then after the study and analyzed at a local laboratory to determine the effect of prunes pulp on plasma lipid-profile consisting HDL, LDL, VLDL and triglycerides (Annoni *et al.*, 1982; Stocker and Keaney, 2004).

Ethical Approval

Ethical approval was taken from the Institute of Bioethical Committee by reference No.258.

STATISTICAL ANALYSIS

Data obtained from each parameter was subjected to an appropriate statistical technique to determine the level of significance (Steel *et al.*, 1997). The software used for the statistical analysis was SPSS 2.0 version.

Table 1: Treatment plan for efficacy study

Groups	Dosage (Prunes Pulp)
G I (Hypercholesterolemia rats)	Normal diet
G II (Hypercholesterolemia rats)	Normal diet +12 gm dried plum (3prunes)
G III (Hypercholesterolemia	Normal diet +24 gm dried
rats)	plum (6prunes)

Table 2: Proximate	composition	of prunes	in percentage
(%)			

Nutrients	Percentage values (%)
Moisture	63.34
Fat	0.1-0.3
Fiber	1.3-2.4
Ash	0.3-0.4
Protein	0.4-0.9
Potassium	0.9-1.4
Magnesium	1.2-1.9

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RESULTS

Following study or work was an effort to inquire the anticholesterol effect of prunes following their mineral analysis. For measuring, prunes were judge for compositional attributes and antioxidative-activity and also effectiveness measure in rat experimental modeling. The physiochemical determination of prunes was done on following characteristics, moisture, fat, fiber, ash and also protein. The orally 12gm and 24gm prunes were provided to hyperlipidemic rats for 2 months study. The following data taken to statistical examination for analyze the level of effectiveness and final effects.

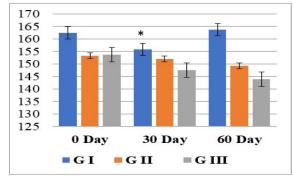


Fig. 1: Plasma cholesterol (mg/dl) level of experimental groups.

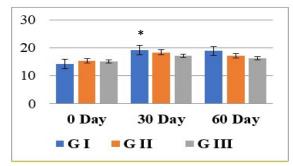


Fig. 2: Body Weight of experimental groups

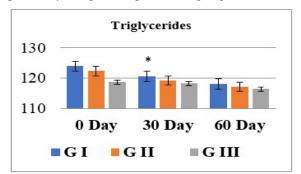


Fig. 4: Level of Plasma triglycerides (mg/dl) in experimental rats.

Proximate analysis

Proximate analysis results exhibit the different percentages of all nutrients including moisture 63.34%,

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ash 0.3-0.4%, fiber 1.3-2.4%, pectin 0.8-1.0%, protein 0.4-0.9% and fat 0.1-0.3%. Minerals include for proximate are potassium0.9-1.4% and magnesium 1.2-1.9% as shown in (table 1). So, it was assessed that prunes had sufficient amount of nutrient. In prunes the percentages of ash, fat, fiber and protein was almost same as described in previous research (Stecewicz-Sapuntzakis, 2013). The difference in values may be due to different environmental circumstances.

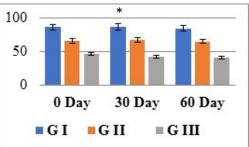


Fig. 5: The level of low-density lipoprotein (mg/dl) in experimental groups

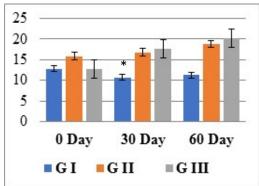


Fig. 6: High density lipoproteins (mg/dl) level observed in experimental groups

Fiber and mineral analysis

Results of fiber (table 2) described fiber content present in prunes. It was observed that two serving containing 24g dried plum have greater amount of fiber than one serving which contain 12g of dried plum. The concentration of fiber was similar to another study conducted by Stecewicz- Sapuntzakis (2013). It was examined that prunes have sufficient amount of potassium and provide magnesium that effective result on hyperlipidemia and helps to control cardiovascular diseases. The analysis of variance of triglycerides were given in table 7.

Efficacy study

Determination of plasma lipid profile

Cholesterol helps in regulating the metabolism of the body also play role in the production of hormones. Promote the integrity of membrane and very essential role in fat digestion. CHOD-PAP method used for the determination of cholesterol level.

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Source	DF	SS	MS	F-Value
Treatment	2	2.758	1.379	0.67°
Day	2	553.954	276.977	133.96 ^a
Treatment*Day	4	29.078	7.269	3.52 ^b
Error	27	55.824	2.068	
Total	35	641.614		

Table 3: Analysis of variance cholesterol

Table 4: Mean +SD of Cholesterol (mg/dL)

Treatment	Days				
Treatment	0	30	60	Mean Value	
GI	162.32	155.83 ^a	163.73 ^a	160.63	
G II	153.24	151.91 ^b	149.26 ^b	151.47	
G III	153.66	147.52 ^c	143.92 ^c	148.37	
Mean Value	156.41	151.75	152.30		
P-Value	0.501	0.020	0.001		
SEM	0.40	0.22	1.06		

NS: Non-significant; *: 0.01<P<0.05; **: 0.001<P<0.01; ***: P<0.001.

SEM: Standard error mean

G I: Normal diet; G II: Normal diet +12 gm dried plum (3prunes); G III: Normal diet +24 gm dried plum (6prunes

Table 5: Analysis of Variance of weight

Source	DF	SS	MS	F-Value
Treatment	2	5.834	2.9169	4.01 ^b
Day	2	141.704	0.8519	97.39 ^a
Treatment*Day	4	2.916	0.729	1°
Error	27	19.642	0.7275	
Total	35	170.096		

Table 6: Mean +SD of Weight

Treatment	0	30	60	Mean Value
GI	14.18	19.15 ^a	19.9 ^a	17.41
G II	15.33	18.36 ^{ab}	17.13 ^b	16.94
G III	15.10	17.15 ^b	16.25 ^c	16.17
Mean Value	14.87	18.22	17.43	
P-Value	0.167	0.010	0.005	
SEM	0.351	0.481	1.220	

NS: Non-significant; *: 0.01<P < 0.05; **: 0.001<P < 0.01; ***: P < 0.001.

SEM: Standard error mean

G I: Normal diet; G II: Normal diet +12 gm dried plum (3prunes); G III: Normal diet +24 gm dried plum (6prunes

Table 7: Analysis of variance of triglycerides

Source	DF	SS	MS	F-Value
Treatment	2	34.34	17.171	0.44 ^b
Day	2	79.26	39.632	1.03 ^a
Treatment*Day	4	32.68	8.171	0.21 ^b
Error	27	1043.31	38.641	
Total	35	1189.61		

Table 8: Mean +SD of triglycerides (mg/dL)

Treatment	Days				
Treatment	0	30	60	Mean Value	
GI	123.91	120.61	118.1 ^a	120.87	
G II	122.3	119.19	117.07 ^b	119.52	
G III	118.66	118.2	116.33 ^b	117.73	
Mean Value	121.61	119.33	117.17		
P-Value	0.487	0.960	0.010		
SEM	1.55	0.669	0.510		

NS: Non-significant; *: 0.01< P<0.05; **: 0.001< P<0.01; ***: P<0.001.

SEM: Standard error mean

G I: Normal diet; G II: Normal diet +12 gm dried plum (3prunes); G III: Normal diet +24 gm dried plum (6prunes)

Table 9: Analysis of variance of low-density lipoprotein (LDL)

Source	DF	SS	MS	F-Value
Treatment	2	8887.5	4443.75	60.28 ^a
Day	2	2907.6	1453.82	19.72 ^b
Treatment* Day	4	572.9	143.22	1.94 ^c
Error	27	1990.4	73.72	
Total	35	14358.4		

 Table 10: Mean +SD of LDL (mg/dL)

	Days				
Treatment	0	30	60	Mean Value	
GI	86.39 ^b	86.87 ^a	84.58 ^a	85.95	
GII	66.23 ^a	66.96 ^b	64.98 ^b	66.06	
G III	46.11 ^c	42.55°	41.08 ^c	43.25	
Mean Value	66.24	65.46	63.55		
P-Value	0.015	0.001	0.001		
SEM	8.70	12.64	5.44		

Table 11: Analysis of variance high density lipoprotein (HDL)

Source	DF	SS	MS	F-Value
Treatment	2	239.98	119.99	95.29 ^a
Day	2	77.54	38.768	30.79 ^b
Treatment*Day	4	159.97	39.994	31.76 ^b
Error	27	34	1.259	
Total	35	511.49		

 Table 12: Mean +SD of HDL (mg/dL)

Treatment	Days			
	0	30	60	Mean Value
GI	12.84 ^b	10.75 ^c	11.26 ^c	11.62
G II	15.86 ^a	16.78 ^b	18.81 ^b	17.15
G III	12.81 ^b	17.60 ^a	20.22 ^a	16.88
Mean Value	13.84	15.04	16.76	
P-Value	0.020	0.001	0.001	
SEM	0.34	7.94	4.55	

NS: Non-significant; *: 0.01<P < 0.05; **: 0.001<P < 0.01; ***: P < 0.001.

SEM: Standard error mean

G I: Normal diet; G II: Normal diet +12 gm dried plum (3prunes); G III: Normal diet +24 gm dried plum (6prunes)

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DISCUSSION

After mineral analysis, prunes were tested for anticholesterol effects. Prunes were measured for composition, antioxidant activity, and efficacy in rat experiments. Prunes were analyzed for moisture, fat, fiber, ash, and protein. SPSS was used to analyze plasma cholesterol levels using 2-way ANOVA to determine dose-time effects. Cholesterol levels differed in all three therapies except GI (control group). Mean data (table 4) showed that group GI on a normal diet had plasma cholesterol levels of 162.32 mg/dl at 0 day, 155.83 at 30 days, and 163.73 at 60 days. Group GII, on 12gm prunes pulp, had plasma cholesterol levels of 153.24mg/dl at 0 day, 151.91 at 30 days, and 149.26 at 60 days, indicating a significant decrease. Group GIII on 24gm prunes pulp had plasma cholesterol levels of 153.66mg/dl at 0 day, 147.52 at 30 days, and 143.92 at 60 days, indicating a decrease. At 30 and 60 days, cholesterol decreased from GI, GII, and GIII. Day 30 showed low cholesterol in GIII and G II and elevated cholesterol in GI. Day 60 showed greater cholesterol in control group GI and reduced cholesterol in G III who had 6 prunes (24gm) diet and then in G II who received 3 prunes (12gm). A study was carried by Ahmed et al. (2010) in which he assess the effect of prunes on hypertensive patients. Prunes juice was fed to hypertensive patients for 60 days, results exhibit significant decrease in plasma cholesterol level. Similarly, further work was conducted by Gallaher and Daniel, (2008) in their research work both declared that treatment of atherosclerosis with prunes powder significantly lower the plasma cholesterol level in the blood.

At 0, 30, and 60 days, rats were weighed using a weight machine. Statistics reviewed the data. GII and GIII lost body weight. At day 30, GII had the highest body weight and GIII the lowest (table 6). At 60 days, rats' body weights gradually decreased, starting with GIII's 24gm dried plum (6 prunes) diet, then G II's 12gm, and finally GI's normal diet without prunes.

In the study, it was noticed that prunes significantly decrease the triglycerides level in both experimental groups. Mean values (table 8) described that the group GI which was on normal diet declared the triglyceride level at 0 day 123.91mg/dl, at 30 day 120.61mg/dl and at 60 day 118.1mg/dl. Group GII which was on 12gm prunes pulp showed the triglyceride level at 0 day 122.3mg/dl, at 30 day 119.19mg/dl and at 60 day 117.07 mg/dl, these values exhibit significant decrease in triglyceride level. Group GIII which was on 24gm prunes pulp presented the triglyceride level at 0 day 118.2 mg/dl and at 60 day 118.66 mg/dl, at 30 day 118.2 mg/dl and at 60 day 116.33mg/dl, these values also report decrease in triglyceride level. At 0 and 30 day the numerically higher level of triglycerides was observed in those group who fed diet 0 prunes G I and lower level of

triglycerides had in G III. At 60 day the level of triglycerides was gradually decreases G I, GII and GIII, respectively. The lower level of triglycerides was observed in G III who fed 6 prunes and then lower level of triglycerides at 60 day had G II who fed that 3 prunes diet. Similarly, Gallaher et al. (2008) reported that Prunus domestica efficiently reduce the area of atherosclerotic lesion by significantly reduce serum triglycerides and plasma cholesterol level when dried plum consumes in the form of dried powder, because of high in soluble fiber and its antioxidant capacity which reduce the oxidative stress and inflammation. A study was directed by Nishi et al., (2013). to analyze the effect of prunes on triglycerides of hyperlipidemic patients and it was noticed that due to its antioxidative property it may scavenge the free radicals and helps in lowering the oxidative stress and also decrease the triglyceride level. Further research was reported by Choi et al. (1991). to check the hypoglycemic effect of prunes methanolic extract of prunus was add in the rat's diet for 6 days and analyze its effect and it was noted that there was a efficiently decrease blood triglycerides and total cholesterol mainly VLDL.

Mean values (table 10) declared that the group GI which was on normal diet showed the plasma LDL level at 0 day 86.39 mg/dl, at 30 day 86.87 mg/dl and at 60 day 84.58 mg/dl, these values exhibit that GI did not showed decrease in plasma LDL level. Group GII which was on 3 prunes (12gm) showed the plasma LDL level at 0 day 66.23 mg/dl, at 30 day 66.96mg/dl and at 60 day 64.98 mg/dl, these values presented significant decrease in plasma LDL level. Group GII which was on 6 prunes (24gm) exhibit the plasma LDL level at 0 day 46.11 mg/dl, at 30 day 42.55mg/dl and at 60 day 41mg/dl, these values of variance in LDL were given in table 9.

At 0 day the level of LDL had higher in G I and lower level of LDL was observed in G II and then G III. The lower level of LDL was observed at 30 in G III and then G II and higher level of LDL had in G I group. At 60 day the low level of LDL was observed in G III who fed diet with 6 prunes and after that the lower level of LDL had G II that received diet with 3 prunes.

A study was conducted by Tinker *et al.*, 1991 to analyze the effect of prunes fiber on hyperlipidemic patients and it was assessed that prunes fiber has significantly lower the LDL level. In another research work treatment of hyperlipidemia was find out by Tinker *et al.*, 1994 and it was declared that plasma LDL level efficiently decrease by prunes. Similarly, Roomi *et al.* 2013 conducted research on polyphenolic extract of *Prunus domestica* on hypolipidemic rats and gave them a monthly dose of 25g/kg body weight of *prunus domestica* red (PDR) for one group and *prunus domestica* yellow (PDY) other group of rats and check the effect on serum lipid profile and concluded that the antioxidant capacity of phenolic compounds significantly decreases the level of VLDL and LDL.

Mean values (table 12) state that the group GI which was on normal diet showed the plasma HDL level at 0 day12.84mg/dl, at 30 day 10.75mg/dl and at 60 day 11.26mg/dl, these values exhibit that GI did not showed increase in plasma HDL level. Group GII which was on 3 prunes (12gm) showed the plasma HDL level at 0 day 15.86mg/dl, at 30 day 16.78mg/dl and at 60 day 18.81mg/dl, these values presented efficient increase in plasma HDL level. Group GIII which was on 6 prunes (24gm) exhibit the plasma HDL level at 0 day 12.81mg/dl, at 30 day 17.60mg/dl and at 60 day 20.22 mg/dl, these values also showed significant increase in plasma HDL level. The values of variance in HDL were given in table 11.

HDL levels at 0, 30, and 60 days were statistically significant. G III had the highest HDL at 0 Day, followed by G II and G I. At day 30, G III and GII had greater HDL levels than G I. GIII with 6 prunes exhibited greater HDL at 60 days, followed by G II with 3 prunes. According to Tinker *et al.* (1994), prunes have a high fiber content that reduces oxidative damage in the blood, lowering LDL and raising HDL later on. That reduces heart issues. Ahmed et al. (2010) treated hypertension individuals with dried plum (prunes) and found that it increased HDL while reduced total cholesterol.

CONCLUSION

It was accomplished that prunes are excellent source of dietary fiber and minerals. Prunes significantly affect the plasma lipid profile, decrease the total cholesterol, LDL cholesterol and triglycerides and improve the levels of HDL cholesterol. Not only dietary fiber also minerals and polyphenols in prunes help in the reduction of cholesterol.

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