

HYPOCHOLESTROLEMIC EFFECT OF PERSIMMON PEEL POWDER IN RABBITS

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ABSTRACT: *The research work was conducted to assess the hypocholesterolemic effect of persimmon peel powder in hyperlipidemia induced rabbits. Rabbits were fed diets to raise cholesterol for 10 days. Then peel was dried in hot air oven and lyophilizer and was converted into powder. The diet of hyperlipidemic rabbits was supplemented with 0, 10 and 20% concentration of oven dried or lyophilized peel powder. After four weeks of this diet therapy, it was observed that the maximum reduction in blood cholesterol level, triglycerides and low density lipoproteins (LDL) was observed in the rabbits fed diets supplemented with 20% peel powder with a mean reduction from 93 mg/dl to 52.25 mg/dl. Whereas, there was an increment in levels of high density lipoproteins with mean increment from, 4.5 mg/dl to 31.375 mg/dl when feed was supplemented with 20% peel powder. It may be concluded that the persimmon peel powder is effective in lowering total blood cholesterol, triglycerides and LDL as a diet therapy eventually reducing the risk of cardiovascular diseases.*

Keywords: Hypocholesterolemic, Persimmon Peel Powder, Rabbits, Diet Therapy

INTRODUCTION

A food is known as functional food, which along with providing sufficient nourishment also affects positively more than one body functions in such a manner that it increases fitness wellbeing and diminishes disease risk. The term nutraceutical and functional food are used interchangeably and are defined as “any substance that is a food or part of food that provides medical and/or health benefits including the prevention and treatment of diseases” [1].

The health benefits of functional foods are ultimately evaluated in controlled clinical and nutritional studies in humans. However, *in-vitro* and animal studies can be used as preamble of human studies and to explain mechanisms [2]. Fruits and vegetables are vital parts of diet as they provide dietary fiber, natural antioxidants and many other bioactive compounds. During processing of various fruits, large quantities of by products are produced which are discarded as waste. Among fruit waste, peels are largest one and these are rich in various bioactive compounds and dietary fiber potentially having different beneficial effects on human [3]. Persimmon (*Diospyros kaki*) is a deciduous fruit, belongs to the Ebenaceae family [4]. It is largely cultivated in subtropical and temperate regions of the world [5]. China, Korea and Japan are the leading world persimmons producers, with an approximate production of 2.5, 0.5 and 0.25 million metric tons per year, respectively [6]. Because of the high bioactive compounds such as ascorbic acid, polyphenols and tannins [7, 8] the persimmons may be associated with various health benefits such as anticarcinogenic, antidiabetic and hypocholesterolemic [7, 9, 10, 11, 12, 13]. Although persimmon peel (PP) is generally regarded as waste but carotenoids and polyphenols concentration is higher in the peel than in the pulp. The phenolic compounds present in peel include p-coumaric, gallic acid, protocatechuic, proanthocyanidins and tannins including catechin, epicatechin, epigallocatechin, catechin-

gallate, galocatechin and galocatechin-gallate [7, 14, 15, 16].

Due to the presence of above mentioned functional compounds, it is claimed that persimmon may possess the potential health benefits such as reversal of multidrug resistance, antitumor [9], antidiabetic [10], hypocholesterolemic and antioxidant [17] and decrease the plasma lipids [18]. Keeping in view the health benefits of persimmon, the importance of waste management and increasing tendency of hypercholesterolemic patients, the present research work was planned for investigating the hypocholesterolemic effects of persimmon peel in rabbits.

MATERIALS AND METHODS

The research work was conducted at Food Microbiology Laboratory, Institute of Food Science and Nutrition, University of Sargodha, Sargodha. Two types (oven dried and freeze dried) of persimmon peel powder were prepared in order to evaluate their nutraceutical value with respect to their hypocholesterolemic effects.

SAMPLE PREPARATION

Persimmon fruit was purchased from local market and then washed with tap water. The twigs of all persimmons were removed with help of knife. The peel was removed and dried in a hot air oven (Binder Company) at 40-50 °C for two days [10] or by lyophilization in a lyophilizer (Christ alpha 1- 4 LD plus, type: 101541) at -50 °C. After drying, the peel was ground to make powder using electronic grinder and the powder was sieved through 40-60 mesh sieves to obtain fine powder which was stored in air tight plastic containers at room temperature for further use [10].

PROXIMATE ANALYSIS OF PERSIMMON PEEL POWDER

The proximate analysis of oven dried and freeze dried persimmons peel powders was performed according to the methods recommended by AOAC [19].

BIOLOGICAL STUDIES

EXPERIMENTAL ANIMALS AND DIETS:

All the research work was conducted on rabbits in Animal house at Department of Pharmacy, University of Sargodha. The experiment lasted for 6 weeks including 14 days of acclimatization, 10 days to raise cholesterol and eighteen days to feed the rabbits with experimental diets. Twenty New Zealand white rabbits of mixed gender of age 8-10 weeks were purchased from local market. These rabbits were housed individually in cages under controlled environmental conditions and hypercholesterolemic diet was prepared to raise blood cholesterol level of rabbits (Table 1). Regular dose of 250g of this diet was given to rabbits in two parts one in early morning and one in afternoon as recommended by Choi *et al.* [20].

After 10 days, to treat the rabbits, five different experimental diets were formulated using 0, 10 and 20% level of oven dried or lyophilized PP (Table 2). Each diet was kneaded well by addition of some distilled water to prepare the pellets used in feed. On the basis of five treatments all twenty hyperlipidemic rabbits were divided into five groups. Each group contained 4 rabbits two male and two female and was fed on separate diet as per treatments. Feed intake for each rabbit was standardized to 250 g/day throughout experiment of four weeks [20].

WEIGHING OF RABBITS

During the experimental period, rabbits were weighed weekly with help of electronic balance (model# D455201437) [20].

BLOOD ANALYSIS

All rabbits were trapped in rabbits stand and blood was withdrawn from jugular vein of all rabbits with help of 3 cc BD syringe and sample was collected in blood collection tubes of 5ml containing Etylenediaminetetraacetic acid (EDTA). To harvest serum, a table top centrifuge at 14000 rpm for 5 minutes was used. Serum was evaluated of cholesterol, triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins (VLDL). Blood collection was done weekly.

DATA ANALYSIS

The data obtained was subjected to statistical analysis of variance technique (ANOVA) under multi factor factorial completely randomized designs (CRD). The P values of <0.05 were adopted as statistically significant. The mean of all treatments were also compared by using LSD test adopting the method as described by [21].

RESULTS

Results regarding cholesterol, TG, HDL and LDL revealed that the effect of concentrations of persimmon peel powder and duration of treatment was significant (P<0.05) whereas the effect of type of peel powder used (oven dried and freeze dried) was found to be non-significant (P>0.05). The interactive effect of PP concentration and treatment time was also significant (P<0.05) while all other first order interactions (powder concentration x powder type, treatment duration x powder type, powder concentration x powder type x treatment duration) showed non-significant (P>0.05) results (Table 4).

The lowest blood cholesterol level and TG contents were observed in rabbits fed on diet supplemented with 20% peel

powder whereas, the highest value was observed in rabbits fed on diet supplemented without persimmon peel powder. The effect of treatment period indicated that with the passage of treatment time there was a significant decline in blood cholesterol and TG contents. The decline in blood cholesterol level was from 91.14 mg/dl to 66.46 mg/dl whereas, TG reduced from 113.46 mg/dl to 87.13 mg/dl during a period of four weeks (Fig. 3).

TOTAL CHOLESTEROL AND TRIGLYCERIDES

Table 1: Composition of Hypercholesterolemic Diet

Ingredients	Quantity (gm/Kg)
Barley	360.00
Corn Starch	360.00
Soya Bean Meal	50.00
Vitamin	15.00
CaCO ₃	20.00
NaHPO ₄	20.00
Salt (NaCl)	5.00
Sugar	100.00
Corn oil	70.00

Table 2: Composition of Experimental Diets

Ingredients	Quantity (gm/Kg)
	T ₀
Barley	380
Corn Starch	370
Soya Bean Meal	70
Vitamin	15
CaCO ₃	20
NaHPO ₄	20
Salt (NaCl)	5
Sugar	100
Corn oil	20
Persimmon peel powder (Oven Dried)	NIL
Persimmon peel powder (Freeze Dried)	NIL

Table 3: Composition of persimmons peels powder (%)

DF	Sum of Squares			
	T. Cholesterol	Triglycerides	HDL	LDL
2	1318.91*	85.56*	1103.16*	830.36*
3	2352.72*	753.78*	2663.03*	1140.69*
1	42.30 ^{NS}	9.63 ^{NS}	24.30 ^{NS}	3.68 ^{NS}
6	535.18*	108.92*	636.75*	290.38*
2	17.49 ^{NS}	3.31 ^{NS}	10.97 ^{NS}	0.97 ^{NS}
3	7.71 ^{NS}	0.40 ^{NS}	13.38 ^{NS}	5.15 ^{NS}
6	4.36 ^{NS}	0.30 ^{NS}	8.68 ^{NS}	1.64 ^{NS}
48	28.49	11.56	13.87	4.51
71				

NS = Non Significant

* = Significant

Table 4.

Parameters	Hot Air Oven	Freeze Dried
Moisture	7.04	7.13
Ash Contents	3.72	3.64
Fat Contents	1.88	2.30
Protein	3.61	3.58
Fiber Contents	40.50	39.01
Cellulose	22.00	19.26
Hemi cellulose	2.15	1.00
Lignin	18.60	17.20

Fig. 1: Effect of persimmon peel powder concentration on total cholesterol and triglycerides

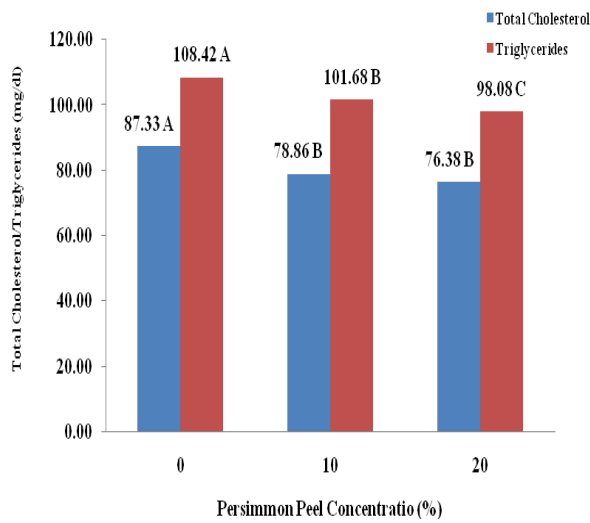


Fig. 2: Effect of treatment period on total cholesterol and triglycerides

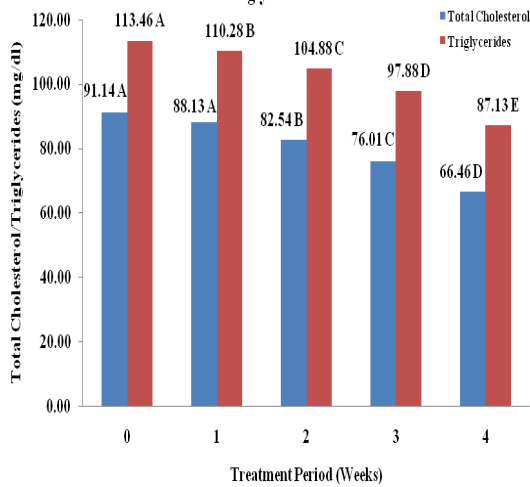


Fig. 3: Combined effect of peel powder concentration and treatment period on total cholesterol and triglycerides

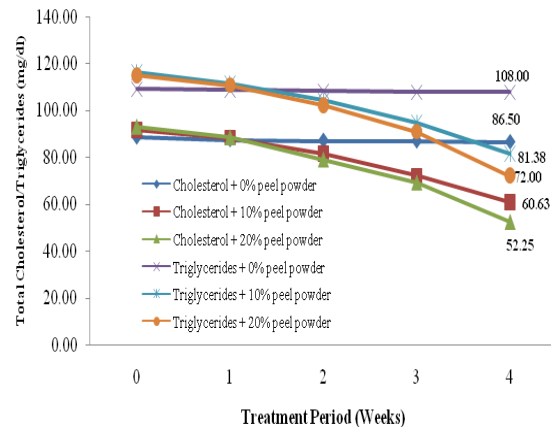


Fig. 4: Effect of persimmon peel powder concentration on HDL and LDL

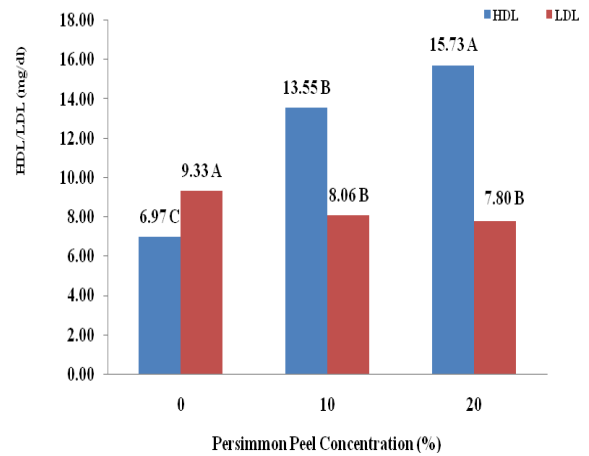


Fig. 5: Effect of treatment period on HDL and LDL

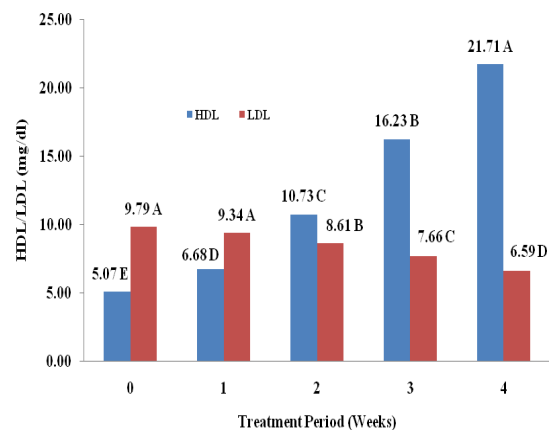
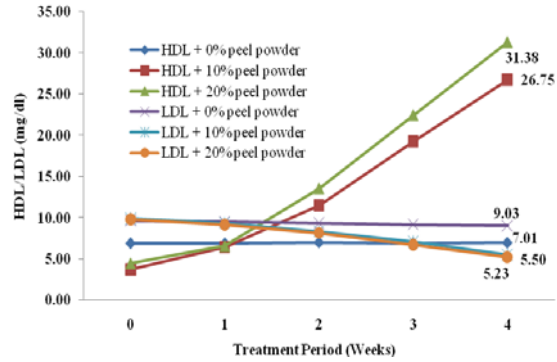


Fig. 6: Combined effect of peel powder concentration and treatment period on HDL and LDL



HGH AND LOW DENSITY LIPOPROTEINS

Results indicated that with increment in concentration of peel powder in the diet there was a gradual increment in HDL and a gradual decline in LDL. The maximum level of HDL was observed in rabbits fed on diet supplemented with 20% peel powder whereas, the minimum level was found in rabbits fed on diet supplemented without persimmon peel powder. Whereas, reverse was true for LDL. The ranges for HDL and LDL were from 6.9 mg/dl to 15.7 mg/dl and 7.8 mg/dl to 9.33 mg/dl, respectively (Fig. 6).

DISCUSSION

Persimmon peel contains, the phenolics including; p-coumaric, gallic acid, protocatechuic, proanthocyanidins and tannins including catechin, epicatechin, epigallocatechin, catechin-gallate, gallic acid, gallocatechin and gallic acid-gallate [7, 14, 15, 16]. In present study the reduction in total cholesterol, TG and LDL might be due to the decrease in oxidative stress by effect of polyphenolic compounds and dietary fiber present in PP as the dietary fiber in presence of antioxidant compounds are effective in reducing blood cholesterol level in rabbits [22]. This might also be related to less assimilation of total cholesterol, TG and LDL as reported by Gorinstein *et al.* [23] while working on rats fed on diet supplemented with persimmon peel. These results further revealed that there were positive effects of PP supplemented diet in lowering total cholesterol, TG and LDL during a period of only 4 weeks. The similar results were observed by Choi *et al.* [20] while studying the hypocholesterolemic effects of herbal medicines on rabbits. The results of present study were also supported by the findings of [24] who observed hypocholesterolemic effects of fruit (*Caranumodontophullum*) parts within a time period of only four weeks in rabbit's blood. Increase in HDL level might be related to the presence of carotenoids and dietary fiber in peel of persimmon as reported by Shakirin *et al.* [22] who found that antioxidants and dietary fiber are effective against cardiovascular diseases as dietary fiber in presence of antioxidant compounds is effective in increasing blood HDL level.

CONCLUSION

From these results it may be concluded the persimmon peel powder is effective in lowering total blood cholesterol, triglycerides and LDL as a diet therapy eventually reducing the risk of cardiovascular diseases.

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