Effects of some legumes on hypercholesterolemia in rats

Sanaa, A.Mahfouz[1];Shahenda, M. Elaby[1] and Hassan,Z.Hassouna[2]

[1]Regional Center for Food and Feed, Agriculture Research Center, Egypt
[2]Food Science & Nutrition Department, National Research Center, Dokki, Cairo, Egypt
Hz.hassouna@hotmail.com

Abstract: This work was undertaken to compare between the effect of diets containing legumes such as white lupin (*lupinus albus*), bitter lupin (*lupinus terms*) and fenugreek seeds (*trigonella foenum-graecum*) on hypercholesterolemia rats. A total of 30 Albino rats divided into 5 groups, each of 6 were used. Twenty four rats were fed hypercholesterolemic diets (positive control), another 6 rats were fed on basal diet (negative control which was similar to diet (positive control) but with no added cholesterol. The rats were fed for 30 days, then decapitated. Fasting serum total cholesterol, HDL-C, VLDL-Cl, triacylglycerol, blood glucose, GOT, GPT, AP, serum protein, serum albumin, serum ceratinine and urea level were measured. The diets containing the three different legume species produced different effects. Diets of bitter lupin and fenugreek seeds were more potent to lower raised serum cholesterol level than diet white lupin. Also, bitter lupin and fenugreek diets were the best to decrease the serum LDL, VLDL- cholesterol and triacylglycerol, while it increased the HDL- cholesterol. In conclusion, it is recommended to utilize these legumes to prepare healthy diets to protect against hypercholesterolemia.
[Sanaa, A.Mahfouz; Shahenda, M.Elaby and Hassan, Z. Hassouna. **Effects of some legumes on hypercholesterolemia in rats**. *J Am Sci* 2012;8(12):1453-1460]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 195

Key words: Hypercholesterolemia, Fenugreek seeds, White lupin ,Bitter lupin.

1. Introduction

Hypercholesterolemia and its implications for cardiovascular diseases is a major problem in human health , and much attention has been paid to dietary intervention . (Anderson *et al.,* 1999). It is confirmed that the plasma cholesterol concentration can be affected by the type of dietary proteins (Cesare *et al.,*2012). Proteins can be divided into two types: one is based on the difference in the amino acid composition and the other is based on difference in the physiochemical properties (Assmaa *et al.,*2002). Several mechanisms were proposed to explain this cholesterol-lowering effect, these involved legume components such as proteins and their amino acid profiles, lipid fractions, fiber, saponins and phytosterols(Kimio *et al.,*1996). Kushi *et al.*(1999) observed that diets high in legumes are beneficial for health and become a topic of scientific interest. There is growing evidence that legumes play an important role in the prevention of diseases. More over the hypercholesterolemic properties of legumes appear to be more effective due to combined effects of several factors acting in concert rather to Single seed constituent. The selective effects of legumes, is simultaneously increasing in high-density-lipoprotein cholesterol anti-atherogenic[(Fruhbeck *et al.,*1997). James *et al.*(2011) reported that a protein-rich legume as lupin, has been shown to have similar effects of those of soy in lowering serum cholesterol levels. Also the lupin kernel (LK fiber) as well as whole lupin seeds has been demonstrated to have a cholesterol-lowering effect in pigs and in man. Lupin Kernel fiber diet reduced total cholesterol , LDL and LDL / HDL ratio and provided favorable changes to serum lipid measures in men (Hall *et al.,* 2005).Fenugreek (*Trigonella foenum - graecum*) is an annual crop belonging to the legume family (Petropoulos ,2002) .According to Lust (1986) fenugreek is one of the oldest known medicinal plants in the recorded history. Basch *et al.* (2003) reported that fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses. Fenugreek is reported to have anti-diabetic, anti cancer, anti-microbial, anti-parasitic and hypocholesterolemic effects (Al-Habori and Raman ,2002). El- Nikeety *et al.* (2010) found that a significant higher amount of soluble, insoluble and total dietary fibercontent in fenugreek and lupine. Therefore, the aim of this study was to determine the chemical composition and amino acids content of seeds of white ,bitter lupin and fenugreek as whole . Also to study the effect of the diet supplemented with these seeds on hypercholesterolemic male albino rats.

2. Material and methods

1- Materials:

- White lupin seeds (*Lupinus albus*) and fenugreek (*TrigoneIIa foenum - graecum*) were obtained from local market of Giza , Egypt.
-Bitter lupin (*lupinus terms*), variety Giza (1), was purchased from the Agricultural Research Center, Ministry of Agriculture Giza, Egypt.

-Casein 85% pure cholesterol and bile salts obtained from Sigma Chemical Co.
-Corn oil and sheep tail fat were burchased from local market.
-Animals: adult male albino rats (30 animals) was purchased from the Medical Center, Giza, Egypt.

Preparation of raw material:

Raw materials (white, bitter lupin and fenugreek whole seeds) were cleaned, washed with tap water and dried at 50 ^{0}C in the oven under vaccum over night. The dried seeds were ground, then kept in polyethylene bags at room temperature until chemical analysis.

Chemical analysis:

Moisture , ash, crude fiber, total proteins, total lipid and total alkaloids, were determined according to A.O.A.C (2000) Amino acids of legumes seeds were determined according to the method of Official Journal of the European Communities 19- 9-98.

Experimental diets:

Diets were formulated as presented in table (1) basal diet (negative diet) prepared as reported by Lane-peter and person
(1971)] and hypercholesterolemic diets (positive diet) as reported by Dabai *et al.*(1996)

Table (1): Formulation of experimental diets (g/100g diets).

Composition of the diets	Basal 1	Basal 2	Diet 3	Diet 4	Diet 5
Casein	25, 000	25.000	-	-	-
White lupin	-	-	54.940	-	-
Bitter lupin	-	-	-	49.620	
Fenugreek	-	-	-	-	81.300
corn oil	10.000	5.000	0.780	2.090	-
Animal fat	-	5.000	5.000	5.000	5.032
Cholesterol	-	1	1	1	1
Bile salts	-	0.500	0.500	0.500	0.500
Mineral mix[1]	4.000	4.000	4.000	4.000	4.000
Vitamin mix[2]	1.000	1.000	1.000	1.000	1.000
Cellulose	5.000	5.000	-	-	-
Starch	55.000	53.500	32.780	36.790	7.168
Total	100	100	100	100	100

Basal 1: Normal control diet(-cont. diets).
Basal 2: Hypercholesterolemic control diet(+cont. diet).
Diet 3: Hypercholesterolemic diet supplemented with white lupin whole seeds.
Diet 4: Hypercholesterolemic diet supplemented with bitter lupin whole seeds.
Diet 5: Hypercholesterolemic diet supplemented with fenugreek whole seeds.
[1]Mineral mix: according to method of Lane – peter and person, (1971).
[2] Vitamin mixture: according to method ofLane- peter and person, (1971).

Design of animal experiment:

Thirty male albino rats, average weight 78 -80 g. were used. Animals were housed individually in wire bottomed cages under standard environmental condition of temperature, relative humidity (55%) and dark /light cycle. Tap water was supplied. Rats were fed on the basal diet for one week then were divided into five groups each of 6 as follows:
-Group (1): Normal rats fed on basal diet (negative diet).
-Other groups; hypercholesterolemia was induced in rats, by feeding on hypercholesterolemic diet according to Dabai *et al.*(1996) and Mona Doweidar (2001).
-Group(2):Hypercholesterolemic rats fed on hypercholesterolemic diet (positive diet) .
-Group (3) Hypercholesterolemic rats fed on hypercholesterolemic diet containing 20 % protein (as whole white lupin seeds) instead of casein.
-Group (4): Hpercholesterolemic rats fed on hypercholesterolemic diet containing 20 % protein (as whole bitter lupin seeds) instead of casein.
-Group (5): Hypercholesterolemic rats fed on hypercholesterolemic diet containing20 % protein (as whole fenugreek seeds) instead of casein.
-Food and water were given *ad- libitum* for 30 days. Food intake and body weight were recorded weekly.

Blood sampling

At the beginning of the feeding period and after 30 days, blood samples were collected from each rat from the territorial venous plexus and were received into clean dry centrifuge tubes to separate serum.

Serum was transferred into dry clean tubes, kept in deep-freezer at -20 c° until biochemical analysis.

Biochemical Analysis:

include determination the following parameters: Serum total cholesterol(according to Thomas, L.1992), HDL-C and LDL-C (Richmonds,N.1973), Triacylglycerol (Fossati,P,1982) , Alkaline phosphates (ALP) activity was measured using the method of (Varley *et al.,* 1980) , serum albumin (Doumas and Biggs ,1971), total protein (Weichsel, B ,1946), Aspartate amino transferase (AST) and alanine amino transferase (ALT) activities (Bergmeyer an Harder,1986) , blood glucose (Trinder,1969) , serum creatinine (Henry,1974),and serum urea (Tabaco,1979).

Statistical analysis:

The obtained data were subjected to statistical analysis using ANOVA test according to the method described by Snedecor and Cochran (1980).

3.Results and Discussion

Chamical composition of legumes:

Table (1) show the values of protein, ether extract, crude fiber, and ash content of legumes (white lupin, bitter lupin and fenugreek seeds). The protein content ranged between 24.60-40.30% , the ether extract was 5.87%- 7.69% , while the crude fibber was 7.72% - 12.49%. The chemical composition of different legumes showed an increase in protein and fiber contents and low carbohydrate content in white lupin and bitter lupin (36.40 and 40.30%) compared with fenugreek. Such findings agreed with those reported by (Erba *et al.,* 2004).

Table (2): Chemical composition and total alkaloid of white lupin, bitter lupin and fenugreek as a whole seeds.

legumes	Proximate composition						Total alkaloids %
	Moisture %	crud Protein%	Ash %	Ether Extract%	Crude Fiber %	N.F.E	
White lupin	6.50	36.40	3.33	7.69	12.49	33.59	0.018
Bitter lupin	6.70	40.30	2.62	5.87	10.43	34.08	0.180
Fenugreek	8.30	24.60	2.92	6.11	7.72	50.35	0.190

N.F.E. (Nitrogen free extract) =(100-moisture+c.protein +ether extract +crude fiber +ash)

Amino acids:

Data presented in table (3) showed that the amount of total essential amino acids varied from 34.64 gm/100gm protein in bitter lupin to 38.52 gm/ 100 gm protein in white lupin, while fenugreek seeds contained the highest amounts of both essential amino acids (41.78 gm/ 100 gm protein) and total amino acids (88.08 gm / 100 gm protein). In the same table methionine and cysteine of white lupin and bitter lupin were lower compared with that fenugreek, this result was confirmed with Agnieszka *et al.* (2005).

Biological assay

Body weight gain: the mean body weight gain of rats over 30 days period are shown in table (4). Rresults indicated that rats in the group fed on negative control diet and bitter lupin diet were significantly lower in body weight gain (79.95 gm and 80.60 gm) compared with positive control group (99.97gm.).

The results showed apparently slight differences in the rates of growth among the groups of rats, this might be related to more or less similar amount of food intake.

The increase in the body weight gain of the group fed on positive control diet might be due to the increased appetite , and food intake. These results are in agreement with that of Atiat *et al.* (1999) , and Dabai *et al.* (1996) who found reduction of gain in body weight of rats fed on legumes compared with hypercholesterolemic control. The decrease in final body weight of the three groups fed on hypercholesterolemic diets supplemented with legumes may be due to increasing legume protein intake and high fiber content . Anderson *et al.* (1996) reported that increasing protein intake may cause weight loss.

Table (3): Amino acid composition of white lupin, bitter lupin and fenugreek as a whole seeds.

Amino acids	White lupin g/100g protein	Bitter lupin g/100g protein	Fenugreek g/100g protein
Essential amino acids			
Isoleucine	3.65	3.50	4.10
Leucine	6.64	6.47	6.26
Lysine	4.03	3.72	5.77
Methionine	0.57	0.54	1.30

Cysteine	1.48	1.58	2.20
Phenylalanine	3.10	2.82	3.78
Threonine	3.29	3.82	3.30
Valine	3.65	3.54	3.82
Arginine	10.19	9.92	9.10
Histidine	1.92	2.55	2.15
T. E. AA	**38.52**	**34.64**	**41.78**
Non essential amino acids			
Glutamic acid	19.69	21.46	16.05
Aspartic acid	9.78	9.60	10.20
Proline	3.48	3.52	3.94
Alanine	3.02	2.77	3.69
Glycine	3.48	3.37	4.75
Serine	4.12	4.04	4.71
Tyrosine	4.12	2.77	2.96
T. Non E.AA	**47.69**	**47.53**	**46.3**
Total amino acids	86.21	82.17	88.08

Table (4): Body weight of experimental animals.

Groups	Initial body weight	final body weight	Gain in body weight
- Cont.	79.50±0.54	159.45±1.84	79.95
+ Cont.	80.25±0.41	180.22±0.89	99.97
Diet3	78.00±0.40	170.50±1.35	92.5
Diet4	81.20±0.79	161.80±1.92	80.60
Diet5	80.50±0.45	164.38±1.82	83.88

All values: means± SE

Serum total cholesterol:

The mean values of serum total cholesterol in table (5) showed that, feeding hypercholesterolemic diet elevated serum total cholesterol to (231.3 mg/dl) and reached to 263.42 mg/dl in positive control group after 30 days of the experimental period, while total cholesterol in negative control group was 102.72 mg/dl. On the other hand, results showed that supplementation diets with legumes which have different protein and amino acids content reduced serum total cholesterol to 205.75 , 209.25 and 216.82 mg / dl for diets containing bitter lupin, fenugreek and white lupin respectively. These finding was similar to that recorded by Jose *et al.*(2005) who reported that plasma total cholesterol concentration was decreased when rats were fed on bitter lupin containing diet compared with control.

Serum lipoprotein:

Data in the same table (5) indicated that rats fed on diets containing a fenugreek, bitter lupin and white lupin respectively, have the highest values of HDL –cholesterol (48.07, 47.0 and 46.65 mg/dl) respectively ,while in case of positive control group the value was (44.00 mg/dl). It's clear from the results that , legumes could resist reduction of HDL-cholesterol in the hypercholesterolemic rats.

The mean serum LDL- cholesterol and VLDL-cholesterol are shown in the same table (5) . The data showed that the mean serum LDL-C and VLDL-C of rats fed on the diets containing legumes were significantly reduced (from 57.37 to 62.60and 99.7 to 108.5 mg/dl) compared with those fed on the positive control diet (91.07) and (133.1 mg/dl) . Data presented in the same table showed that positive control group had a highly increased values of LDL-C (91.07mg/dl) compared with the negative control group (29.82mg/dl). All groups of rats fed on diets containing legumes showed a significant decrease in LDL- C compared with the positive control group . The level of LDL-C of rats fed bitter lupin diet was most lower compared with other groups .The finding in Table (5) indicated that the highest increase in VLDL-C was found in the group of rats in the positive group (133.1mg/dl) compared with negative control group . The level of VLDL-C in rats fed the legume diets(bitter lupin) had decreased in values (99.7 , 101.8 and 108.5mg/dl)compared with positive control group.Huff&Telford,(1985),Kingman,(1991) and Andersson & Major,(2002) reported that the LDL-cholesterol reduction observed by feeding

legumes or their fractions to hypercholesterolemic subjects could result from reduced LDL synthesis and / or increased LDL metabolism. On the other hand Cesare *et al.* (2004) reported that a frequently used dosage of total lupin protein extract from white lupin seeds , reduced total plasma VLDL-LDL cholesterol . Also , Atta Elmnan *et al.* (2012) reported that fenugreek seed had a significant decrease on plasma total cholesterol , triaceylglycerol and LDL. However, a numerical increase was observed in HDL.

Table (5): Serum total cholesterol concentrations and cholesterol contents of serum HDL, LDL and VLDL of experimental rats.

Groups	Total cholesterol			HDL- cholesterol			LDL - cholesterol			*VLDL- cholesterol		
	Initial time	After 30 days		Initial time	After 30 days		Initial time	After 30 days		Initial time	After 30 days	
	mg/dl	mg/dl	%	mg/dl	mg/dl	%	mg/dl	mg/dl	%	mg/dl	mg/dl	%
- Cont.	98.8±0.69	102.72±1.92	100.0	46.0±1.67	53.42±1.50	100.0	28.0±0.71	29.82±0.23	100.0	24.8	19.48	100.0
+Cont.	231.3±0.57	263.42±5.65	256.4	45.5±0.47	44.0±1.77	82.3	69.0±1.37	91.07±3.11	305.6	116.8	133.1	683.2
Diet 3	230.3±2.91	216.82±5.30	211.1	44.8±0.69	46.65±1.78	85.4	69.3±0.44	62.60±0.41	209.9	116.2	108.5	556.9
Diet 4	228.4±1.46	205.75±4.25	200.3	45.3±0.89	47.0±0.71	90.9	68.2±0.45	57.37±1.11	192.4	119.5	99.7	511.8
Diet 5	229.9±0.22	209.25±3.54	203.7	45.3±0.37	48.07±0.36	86.2	38.5±0.77	61.32±0.62	205.6	115.6	101.8	522.5
LSD												
at 0.05		22.85 at P ≤ 0.05			4.71 at P ≤ 0.05			5.28 at P ≤ 0.05				
at 0.01		22.85 at P ≤ 0.01			6.51 at P ≤ 0.01			7.30 at P ≤ 0.01				

*VLDL= total cholesterol-[HDL+LDL] cholesterol

Serum triacylglycerol:

Table (6) shows the mean values of serum triacylglycerol of rats at initial time and after 30 days. The mean serum triacylglcerol levels of rats in the positive control group (102.97 mg/dl) was grater than that of the negative group (59.15 mg/dl) and those of rats fed on legume diets (ranged 84. 40 to 88.05 mg/dl). Therefore a significant reduction in serum triacylglycerol was found that is in agreement with Xue *et al.* (2007) who reported that rats fed on diets containing *Trigonella fonum- graecum* extract, had lower blood glucose, triaceylglycerol and total cholesterol, also Fatima *et al.* (1995) found that ,the mean plasma triacylglycerol level of control group(hypercholesterolemic rats)were greater than that of rats fed on the legumes diets.

Blood glucose levels:

Table (7) show mean blood glucose values for rats fed on either of the three legume diets and control. The mean blood glucose of rats fed bitter lupin diet tended to be that of lower (119.0 mg/dl) than rats fed on the other legume diets (ranged 122.0-123.0 mg/dl) and positive control diet (129.0 gm/dl).The decrease in blood glucose is due to the effect of dietary fiber from the legumes. These results are agreed with Adam (2003) and Patil (2009) .The beneficial effect of fenugreek seed mucilage is due to some of the bioactive compounds present in mucilage, 4-hydroxy isoleucine is an unusual amino acid as anti dyslipidemic and antihyperglycemic agent(Tadigoppula *et al.*, 2006).

Table (6): Serum triaceylglycerol concentrations of experimental rats.

Groups	Serum triglyceride		
	Initial time mg/dl	After 30 days	
		mg/dl	%
- Cont.	52.1±2.06	59.15±2.30	100.00
+ Cont.	92.2±4.08	102.97±3.14	184.35
Diet 3	98.3±2.99	88.05±1.98	155.37
Diet 4	97.6±1.72	84.40±1.85	149.46
Diet 5	98.8±1.04	87.82±1.71	155.77
LSD			
at 0.05		32.27 at $P \leq 0.05$	
at 0.01		44.61 at $P \leq 0.01$	

Table (7): Blood glucose contents of experimental rats.

Groups	Blood glucose contents		
	Initial time mg/dl	After 30 days	
		mg/dl	%
- Cont.	98.0±0.35	100.0±1.06	100.0
+ Cont.	126.0±0.61	129.0±1.82	129.0
Diet 3	127.3±1.08	123.0±2.04	130.0
Diet 4	126.0±0.74	119.0±0.89	119.0
Diet 5	127.0±0.94	122.0±0.61	122.0
LSD			
at 0.05		4.87 at $P \leq 0.05$	
at 0.01		6.74 at $P \leq 0.01$	

Serum of GOT, GPT and ALP:

Data presented in table (8) shows that there were significant changes in the impact of all legumes diets on both of serum GOT(AST), GPT (ALT)and AP, which were (56.0, 40.0 and 220 U /L) compared with rats of the positive group (69.0, 51.0 and 241 U/L values of Serum GOT, GPT and AP were the lowest in rats fed on bitter lupin and fenugreek seeds. These findings confirmed, the healthy roles of legumes in lowering the GOT and GPT activities (Eidi, 2007).

Table (9) showed the mean values of serum total protein and serum albumin of the rats fed different types of legumes diets. It was found that there was a slightly significant difference of serum total protein , whereas serum albumin of rats fed on legumes diets showed non significant changes. These results agreed with those of Gloria *et al.* (2010) who reported that hypercholesterolemia had no significant effect on serum albumin in the rat models.

The results in Table (10) showed that non significant changes in serum creatinine, whereas serum urea were significantly decreased in rats fed on lupin and fenugreek seeds compared with other groups. These results are in concordance with Tharanathan and Mahadevamma (2003) who showed that a high fiber diet seemed to that of lower the urinary phenol and cresol concentration in human.

Table (8): activities of serum glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and alkaline phosphatase (AP) of experimental rats.

Groups	Serum GOT (AST)			Serum GPT (ALT)			Serum alkaline phosphatase (AP)		
	Initial time U/l	After 30 days		Initial time U/l	After 30 days		Initial time U/l	After 30 days	
		U/l	%		U/l	%		U/l	%
- Cont.	47.0±1.08	50.0±2.84	100.0	28.0±0.61	30.0±1.78	100.0	188.0±0.99	188.0±2.70	100.0
+ Cont.	63.0±0.54	69.0±2.48	138.0	45.0±0.54	51.0±0.71	170.0	233.0±1.35	241.0±3.04	127.9
Diet 3	62.0±0.65	60.0±0.89	120.0	45.0±0.74	43.0±0.54	143.0	231.0±0.70	228.0±0.87	120.8
Diet 4	59.0±0.61	56.0±1.27	112.0	44.0±0.54	40.0±0.75	133.0	228.0±0.74	220.0±1.67	116.8
Diet 5	60.0±0.94	59.0±1.62	118.0	45.0±0.71	42.0±0.61	140.0	230.0±0.94	225.0±1.59	119.4
LSD									
at 0.05		6.82 at $P \leq 0.05$			3.44 at $P \leq 0.05$			9.12 at $P \leq 0.05$	
at 0.01		9.44 at $P \leq 0.01$			4.76 at $P \leq 0.01$			12.62 at $P \leq 0.01$	

Table (9): Values of serum total protein and albumin of experimental rats.

Groups	Serum total protein			Serum albumin		
	Initial time g/dl	After 30 days		Initial time g/dl	After 30 days	
		g/dl	% relative		g/dl	% relative
- Cont.	7.41	7.60	100.0	3.90	3.95	100.0
+Cont.	8.06	8.35	109.8	4.10	4.30	108.8
Diet 3	8.1	8.20	107.9	4.10	4.20	106.3
Diet 4	7.9	8.05	105.2	4.20	4.02	101.7
Diet 5	8.0	8.20	107.9	4.10	4.22	106.8
LSD						
at 0.05		0.33 at $P \leq 0.05$			Non significant	
at 0.01		0.45 at $P \leq 0.01$				

Table (10): Serum ceratinine and urea levels of experimental rats.

Groups	Serum ceratinine			Serum urea		
	Initial time mg/dl	After 30 days		Initial time mg/dl	After 30 days	
		mg/dl	% relative		mg/dl	% relative
- Cont.	0.56	0.60	100.0	32.0	35.0	100.0
+Cont.	0.63	0.65	108.3	42.5	46.5	132.8
Diet 3	0.62	0.61	101.6	42.0	41.6	117.1
Diet 4	0.62	0.59	98.3	41.6	42.2	120.5
Diet 5	0.61	0.60	100.0	42.1	40.0	114.2
LSD						
at 0.05	Non significant				6.51 at $P \leq 0.05$	
at 0.01					8.98 at $P \leq 0.01$	

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