

EFFECT OF MUNG BEAN AND RED LENTILS DIETARY FIBER (RAW VERSES COOKED) ON THE LIPID PROFILE OF INDUCED HYPERCHOLESTEROLEMIC RATS

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ABSTRACT: The study was carried out to evaluate the hypocholesterolemic effect of crude dietary fiber extracts of raw and cooked (whole) mung bean (*Vigna radiata*) and red lentils (*Lens Culniras*). High fiber diets were prepared using 35 % crude dietary fiber extract of both raw and cooked mung bean and red lentils. Hypercholesterolemia was induced in 40 sprague dawley male rats, weighing 205-225 g. The animals were equally divided into 5 groups to study the effect of purified diet AIN-93 M, raw and cooked mung crude fiber diet, raw and cooked red lentils crude fiber diet on total cholesterol, very low-density lipoprotein cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol and triglycerides levels. The rat group induced with hypercholesterolemia was fed on purified diet AIN-93-M and labeled as Group I. Group II comprised of induced hypercholesterolemic rats given raw mung crude fiber diet; whereas Group III consists of induced hypercholesterolemic rats that were given cooked mung crude fiber diet, Group IV consisted of hypercholesterolemic rats given raw red lentils crude fiber diet and Group V comprised of hypercholesterolemic rats fed on cooked red lentils crude fiber diet. The hypercholesterolemic group fed on raw mung bean crude fiber diet had significantly lowered the total cholesterol. Likewise, a decrease in very low-density lipoprotein cholesterol and low-density lipoprotein cholesterol with a higher ratio of high-density lipoprotein cholesterol was recorded in the same group. The results are of the view that cooking may have slightly reduced the hypocholesterolemic effect of the pulses under study.

Key words: Crude fiber diet, Hypercholesterolemia, Mung bean, Red lentil.

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INTRODUCTION

The diet of a large number of people especially of lower socio-economic strata in Pakistan is predominantly based on cereals and pulses. A major portion of calories and protein is obtained from a combination of cereals and pulses, the foods that are economical and widely available (Miller *et al.*, 2017 and Hall *et al.*, 2017).). Pulses and lentils are cooked in a thick soup known as 'daal' and are consumed with bread. Two of the most commonly used pulses are mung bean and red lentils; both of these are commonly eaten in whole seed form (Shah *et al.*, 2011).

Mung bean (*Vignaradiata*) is one of the important 'kharif' pulses of Pakistan and is widely consumed in South East Asian countries. It is known as 'dal moong' in the native language (Joshi, 2016). It belongs to genus Phaseolus to Vigna and Fabaceae family. (Singh *et al.*, 2013).

Lentils (*Lens Culniras*) is a 'Rabi' crop and has a small flat disc or lens like seed. It comes in various colors is mostly found in brown or green in color. Like Mung beans, Lentils are also used in two forms whole, (with coat) and de-hulled (without coat) in various different foods around the world (Nosworthy *et al.*, 2018)

The present study aims to see the effect of high crude fiber diets prepared from these pulses on the lipid profile of the hypercholesterolemic rats.

MATERIALS AND METHODS

The NM-92 variety of Mung bean and Masoor-2006 of red lentil were used in this study. Both of these varieties were obtained from NAIB Faisalabad (Nuclear Institute for Agriculture and Biology). The samples from both of these pulses were cleaned, and each sample was divided into two portions of raw and cooked samples. The raw samples were ground coarsely and fiber was extracted through sieving with 20-mm mesh sieve. For the cooked samples the seed were boiled at 100⁰ C for 25 minutes to until tender. Water was drained out to get samples air-dried. The samples were then oven-dried and stored in air tight jars for their subsequent use.

Proximate Analysis: Proximate analysis of each sample to be assayed was done according to the AOAC methods (2005). The estimation of crude dietary fiber was done according to the Weende Method (Czerkawski, 2013), cellulose was estimated by Kurschner and Hank method

(Usman *et al.*, 2010) and lignin by the method as evolved by Fiore *et al.* (2014) (Table -1).

Formulation of Diets: Purified diet (PD) AIN- 93-M was used as the normal rat diet; whereas, the experimental diet was made by modification of the normal rat diet. Crude fiber diet (CFD) extract from raw and cooked whole mung bean and red lentils was used to make the experimental diets computed on 35% crude fiber basis.

Induction of hypercholesterolemia: Hypercholesterolemia was induced in all the experimental groups by feeding them on a mixture prepared from cholesterol powder (Sigma Aldrich), bile salt and dried bovine fat was added to the normal rat diet in the percentage of 2%, 0.25% and 5%, respectively (Aggarwal, 2010). The experimental diets were given to the animals for a period of two weeks to induce hypercholesterolemia in them that was confirmed through test at the end of this period.

Experimental Animals: Male Sprague dawley rats (n=40), aged at 7-10 months and weighing 205-225 g, were kept in an animal room set at temperature of 24°C-28°C. During the first week of experiment, the animals were given no treatment so as to acclimatize them.

The rats were then divided into four experimental groups of 8 rats each; whereas, one group on normal diet served as control. Each group was fed on its respective diet with clean water served *ad libitum* for the experimental period of four weeks. The average feed intake per rat and weight gain by each rat group was also recorded weekly.

Biochemical Assay: At the end of the assigned time of the experiment the animals were given anesthesia of chloroform. The samples of blood were obtained from these animals by cardiac puncture. The blood was drawn slowly from the heart ventricle by a 19 to 25G needle with 1 to 5 ml syringe to refrain from heart collapsing due to shock (Parasuraman *et al.* 2010). These samples of rat blood were saved in plastic tubes containing ethylenediamine tetra acetic acid (EDTA), dipotassium salt, 0.8mg/ml of blood and centrifuged at 1500x g at the temperature of 4° C for 30 minutes to get plasma from the blood samples. Once the plasma was separated these samples were then stored at -70 °C. Later the cholesterol level of these plasma samples was analyzed through enzymatic calorimetric procedures using Sigma diagnostic kit 352. The triglyceride percentage in the samples was estimated through Gilford diagnostic kit 23422).

Five different blood pools were made of each group. Every blood pool was made by adding 0.5 ml of blood was drawn from each of the 6 animals present in an individual group. Proteases inhibitor, epsilon-amino caproic acid (1.3/mg/ml of plasma) and anti-microbial agent (garmicine 50mg/ml, 10 µl/ml of plasma were added to the pooled plasma samples as preservatives. A sample of 1 ml was taken from pooled plasma of each group and

was fractionated by density-gradient ultracentrifugation for the estimation of lipoproteins (Tremblay *et al.* 2004). Later background density of the sample was adjusted to 1.019 with NaCl, plasma was centrifuged at 40,000 rpm for 18 hours at 17 °C into 2ml tube with adapters in a Beckman 50.3 rotor (Beckman, Palo Alto CA). The top 0.34 ml layer, containing the VLDL fraction, was removed with a Pasteur pipette. The next 0.34 ml layer was removed as background; the subnatant density was adjusted to 1.063, and centrifuged at 40,000 rpm for 24 hours 70 C. The top 0.34 ml was removed as the LDL fraction; another 0.34 ml layer was removed as background. The subnatant contained HDL. Lipoprotein fractions were analyzed for cholesterol using the procedure described for plasma. The triglycerides were determined using the colorimetric method (Barker *et al.*, 2005; Hamid *et al.* 2017)

Statistical analysis: The analysis was done using the statistical software SPSS version 20. Multiple comparisons of means were made by one-way ANOVA test.

RESULTS AND DISCUSSION

Chemical composition

a) **Proximate composition:** The results of the raw and cooked mung bean samples respectively were as following moisture 8.99 %vs 7.87%, ash 2.84 %vs 2.12%, protein 22.02% vs 21.33%, fat 2.59% vs.1.60%, crude fiber 4.26% vs 3.98%, cellulose 2.45% vs 1.87% and lignin1.67% vs 1.11% (Table 1). Martens, *et al.* (2013); Shaheen *et al.* (2012) reported the nutritional composition of whole raw mung beans as follows: moisture content 8.25%, ash 4.63%, fat 1.35%, crude protein 25.00%, crude fiber 1.68% and nitrogen free extract 59.09%. The results of study, for certain parameters appeared to be comparable to the above studies. Similar results were also reported by Dahiya *et al.* (2015).

The results of raw and cooked red lentils were as follows : moisture 6.29%vs 5.98%,ash 3.90% vs 3.00% , protein 24.09% vs 22.56% , fat 1.26% vs 1.00%, crude fiber 3.27% vs 2.98%, cellulose 2.26% vs 1.92% and lignin 1.54 %vs 1.02%.Nevertheless (Boyle *et al.* (2010) ; Zia-Ul-Haqet *et al.* (2011) in a similar study reported the crude protein content in whole red lentils (dal masoor sabut) to be within 26.1% and 28.80 % , crude fiber content was reported to be within 6.99% and 8.14%. The fat content ranged from 1.93 to 2.15% and carbohydrates were determined to be within 54.08% to 55.81%.

Effect of Cooking: Since pulses and legumes must be cooked before consumption, hence it is important to see the effects of cooking on their nutritional composition. It was observed that there was some loss of nutrients in both mung bean and red lentils due to cooking (Table 2). Wang *et al.* (2010) reported that cooking decreased the

fiber and overall protein percentage in chickpeas. Similarly Boye and Pletch (2010) observed a minor decrease in protein content, but improved digestibility of pulses due to cooking Campos-Vega *et al.* (2010) also observed a slight decrease overall, in moisture, protein, fat, crude fiber and ash content in the cooked and dried sample of mung bean as compared to raw samples.

Lipid Profile: After the induction of hypercholesterolemia, no significant difference was noted in the total cholesterol (TC) level of rats in all the five groups as all the groups had high level of plasma TC (Table 3). All the experimental rats were fed on their respective diets for 6 weeks. The TC was recorded twice in each group before and after treatment. In the post-treatment, mean TC of the group fed on PD AIN-93-M was significantly ($p < 0.05$) higher than in the hypercholesterolemic groups fed on high fiber diets i.e. from 289.87 mg/dL to 294.34 mg.

The raw mung bean crude fiber diet (CFD) reduced the TC to 256.23 mg/dL from 288.33 mg/dL, which was significantly ($p < 0.05$) higher level as compared to the rest of groups. On the contrary, no significant difference ($p > 0.05$) was found in TC reduction between the groups of rats fed on cooked mung bean CDF (292.09 mg/dell to 266.12dl) and raw red lentils CDF (from 288.22 to 267.21). Hypercholesterolemic group fed on cooked red lentil CFD had significantly less reduction in the mean TC (from 280.12 to 262.02) as compared to the three other groups fed on CFD. While investigating the effect of oat dietary fiber on serum cholesterol level Othman *et al.* (2011) noted that lignin and dietary fiber content was responsible for cholesterol-lowering effect. Likewise, it was noted that crude dietary fiber from different sources lowered the serum cholesterol in hamsters (Makki *et al.*, 2018). There is a decrease in the TC of the group fed on Raw Mung Bean CDF was from which was significantly ($p < 0.05$) higher than the other groups.

No significant ($p < 0.05$) difference was observed in the VLDL level in all the pre-treatment hypercholesterolemic groups (Table 3). However, in the post-treatment groups the hypercholesterolemic group fed on PD AIN-93-M had significantly ($p < 0.05$) higher VLDL level (159.99 mg/dL to 62.38mg/dL). Nevertheless, no significant difference was observed for mean VLDL levels in hypercholesterolemic rats fed on cooked mung bean (59.33mg/dL to 50.45mg/dL) cooked red lentil and raw red lentil CDF (60.43mg/dL to 55.90mg/dL). Nevertheless, mean VLDL level in the hypercholesterolemic rats reared on raw mung bean CD ranged from 58.33 mg/dL to 47.31 mg/dL which was significantly ($p < 0.05$) lower as compared to the other experimental groups (Table 3) observed that a prolonged use of both soluble-fiber i.e. β -glucan and crude insoluble fiber obtained from barley can reduce cardiovascular disease (CVD) risk factor which was a result of a con-

sistent use of CFD which lower the VLDL level to a notable degree.

The group of hypercholesterolemic rats fed on PD AIN-93-M had significantly higher LDL in comparison to the other hypercholesterolemic groups (Table 3). No significant difference was observed in the mean LDL level of rat groups fed on raw mung bean, cooked mung bean and raw red lentils CFD. Nevertheless, hypercholesterolemic rats group fed on cooked red lentil CFD had significantly ($p < 0.05$) higher LDL level as compared to the rest of experimental groups. Marriott *et al.* (2010) noted that contrary to the refined sugar the intake of crude dietary fiber decreased the level of serum LDL level which in turn reduced the risk of CVD's. Hanson *et al.* (2013) who noted that total serum cholesterol (TC) and LDL-cholesterol levels in rats fed on pulse-based diets were lower as compared to the control group. They concluded that pulses had reduced the value of circulating total serum cholesterol and LDL levels in the experimental group.

The induced hypercholesterolemic rats group fed on PD AIN-93-M significantly ($p < 0.05$) lower HDL (75.88mg/dL to 74.31mg/dL) as compared to the rest of hypercholesterolemic groups. Whereas, the hypercholesterolemic group fed on raw mung bean CDF had significantly ($p < 0.05$) higher HDL (76.22 mg/dL to 65.49mg/dL) as compared to the rest of hypercholesterolemic groups. However, no significant difference was observed in the mean HDL level of rat groups fed on cooked mung bean (77.90 mg/dL to 72.00 mg/dL), raw red lentil CDF (77.22 mg/dL to 63.5.16 mg/dL) and cooked red lentil CFD (74.56 mg/dL to 69.56 mg/dL). In a study, conducted on mildly hypercholesterolemic men and women that the subject who consumed higher percentage of dietary fiber from various food had a higher percentage level of HDL as compared to VLDL, and LDL (Tighe *et al.*, 2010). These results are in line with the present study.

The hypercholesterolemic rat group fed on normal rat diet PD AIN-93-M had the highest level of triglycerides (312.55 to 320.12). However, the induced hypercholesterolemic group of rats reared on cooked red lentil CFD had significantly higher ($p < 0.05$) triglycerides level (330.23 mg/dL to 326.4.22 mg/dL) as compared to the groups fed on raw mung bean CDF (326.45 mg/dL to 298.77), cooked mung bean CDF (335.28 mg/dL to 306.12 mg/dL) and raw red lentil CFD (320.32 to 300.87). However, non-significant difference ($p > 0.05$) was recorded in the mean triglycerides level in rat groups fed on raw mung bean, cooked mung bean and raw red lentils CFD.

Al-Tibi *et al.* (2010) recorded the hypocholesterolemic effect of raw whole lentils, cooked whole lentils, raw de-hulled lentils and cooked de-hulled lentils on experimental rats. They observed no significant ($p > 0.05$) difference in serum HDL cholesterol, triglycerides, total

serum cholesterol and LDL cholesterol in the Raw CFD and Cooked CFD groups. Similar trends were also observed in the present study, since no significant differ-

ence was found in the triglycerides level of the hypercholesterolemic rats groups fed on whole cooked and raw red lentils CFD.

Table 1: Proximate composition of mung bean and lentils (g/100g)

Parameters	Raw mung	Cooked mung	Raw lentil	Cooked lentil
Moisture	8.99±0.24	7.87±0.67	7.29±0.51	6.98±0.71
Ash	2.84±0.09	2.12±0.12	3.90±0.35	3.00±0.21
Protein	22.02±0.58	21.33±0.54	24.09±0.31	22.56±0.50
Fat	2.59±0.03	1.60±0.39	1.26±0.21	1.00±0.87
Crude Fiber	4.26±0.40	4.26±0.40	3.27±0.25	2.98±0.17
Cellulose	2.45±0.48	1.87±0.40	2.26±0.16	1.92±0.34
Lignin	1.67±0.26	1.11±0.21	1.54±0.34	1.02±0.19

Means within a row followed by different letters are significantly different at P<0.05 level

Table 2: Composition of experimental diets.

	Cornstarch	Casein (85% protein)	Dextrinized corn starch	Sucrose	corn oil	Crude fiber	Mineral mix	Vitamin mix	L- Cystine	Choline bitartrate	Tert-butylhydroquinone
PD AIN-93-M	465.692	140.000	155.000	100.00	40.00	50.000	35.000	10.000	1.800	2.500	0.008
Raw Mung Bean CFD	165.692	140.000	155.000	100.00	40.00	350.00	35.000	10.000	1.800	2.500	0.008
Cooked Mung Bean CFD	165.692	140.000	155.000	100.00	40.00	350.00	35.000	10.000	1.800	2.500	0.008
Raw Red Lentil CFD	165.692	140.000	155.000	100.00	40.00	350.00	35.000	10.000	1.800	2.500	0.008
Cooked Red Lentil CFD	165.692	140.000	155.000	100.00	40.00	350.00	35.000	10.000	1.800	2.500	0.008

PD AIN-93-AIN: Purified Diet AIN-1993, CDF: Crude Fiber Diet.

Table 3: Effect of various experimental diets on the lipid profile of rats.

Parameters Studied	Pre-Treatment	Post-Treatment
Total Plasma Cholesterol (TC)		
PD AIN-93-G	289.87±3.90	294.34±3.76a
Raw Mung Bean CFD	288.33±3.44	256.23±3.18b
Cooked Mung bean CFD	292.09±9.24	266.12±2.16c
Raw Red Lentil CFD	288.22±8.44	267.21±1.09cd
Cooked Red Lentil CFD	280.12±2.24	262.02±1.08d
Very Low Density Lipoproteins (VLDL)		
PD AIN-93-G	59.99±1.44	62.38±2.11a
Raw Mung Bean CFD	58.33±1.77	47.31±2.43b
Cooked Mung bean CFD	59.33±0.98	50.45±2.34c
Raw Red Lentil Fiber Diet	62.12±1.82	53.21±1.21bc
Cooked Red Lentil CFD	60.43±3.87	55.90±5.87c
Low Density Lipoproteins (LDL)		
PD AIN-93-G	75.88±4.12	74.31±2.99a
Raw Mung Bean CFD	76.22±1.24	65.49±0.38b
Cooked Mung bean CFD	77.90±2.55	72.00±1.99c
Raw Red Lentil CFD	77.22±2.21	63.5.16±1.50b
Cooked Red Lentil CFD	74.56±5.64	69.56±2.11b

High Density Lipoproteins (HDL)		
PD AIN-93-G	138.66±2.77	130.13±2.99a
Raw Mung Bean CFD	133.40±2.21	144.16±2.54b
Cooked Mung bean CFD	138.45±1.44	141.90±2.55b
Raw Red Lentil CFD	136.12±1.71	142.72±2.12b
Cooked Red Lentil CFD	139.89±2.44	140.33±3.66b
Triglycerides		
PD AIN-93-G	312.55±2.55	320.12±2.44a
Raw Mung Bean CFD	326.45±3.34	298.77±2.47b
Cooked Mung bean CFD	335.28±3.66	306.12±2.55b
Raw Red Lentil CFD	320.32±2.52	300.87±2.23b
Cooked Red Lentil CFD	330.23±2.33	326.4.22±1.55c

Means within a row followed by different letters are significantly different at P<0.05 level

Conclusion: A significant reduction was observed in the total cholesterol level of hypercholesterolemic rat groups fed on raw mung bean, cooked mung bean, raw red lentils and cooked red lentils CFD. Similarly, a decrease in VLDL and LDL levels was also recorded in the hypercholesterolemic rat groups fed on raw mung bean CFD. The hypercholesterolemic rat groups reared on raw mung bean CFD was found to be most effective in lowering their triglycerides level. Hence, the study concludes that raw mung bean CFD was most effective in lowering the total cholesterol and triglycerides levels in the hypercholesterolemic rats as compared to cooked mung bean, raw red lentils and cooked red lentils CFD.

The study further concludes that raw mung bean CFD is even more effective in reducing the plasma cholesterol, VLDL, LDL and triglycerides levels in the hypercholesterolemic rats as compared to the cooked mung bean, raw red lentils and cooked red lentils CFD. The study, therefore, indicates that the process of conventional cooking (boiling) at homes may enhance the digestibility of the whole pulses and lentils but at the same time may have a slight inverse effect on their hypocholesterolemic effect.

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