How to Cite:
Rabeh, N. M., Refaat, O. G., & Dhaher, A. G. A. E. (2022). Effect of psyllium seeds and its husk on hypercholesterolemic rats. *International Journal of Health Sciences*, *6*(S1), 116-124. https://doi.org/10.53730/ijhs.v6nS1.4750

Effect of Psyllium Seeds and Its Husk on Hypercholesterolemic Rats

Naeem M Rabeh
Nutrition and Food Science Dept, Faculty of Home Economics, Helwan University, Cairo, Egypt

Omnia G Refaat
Nutrition and Food Science Dept, Faculty of Home Economics, Helwan University, Cairo, Egypt

Aya G Abd El Dhaher
Nutrition and Food Science Dept, Faculty of Home Economics, Helwan University, Cairo, Egypt

***Abstract*---** This study was performed to investigate the outcome of psyllium seeds and their husk on lipid profile for 8 weeks of hypercholesterolemic rats. A number of 36 mature male rats were split into two groups. The first six rats in the study were fed a standard diet (served as – ve control group). The second main group (30 rats) was fed on high cholesterol diet to induce hypercholesterolemia for six weeks, then was divided as follows: Subcategories (1) was fed on a diet with high cholesterol (as a positive control group). Subcategories (2 and 3) were on a diet with high cholesterol supplemented with 2.5% and 5% Psyllium husk powder, respectively. Subcategories (4 and 5) were fed on high cholesterol diet supplemented with 2.5 % and 5% Psyllium seed powder, respectively. The results revealed that liver functions were found to be significantly decreased (P<0.05) by Psyllium husk or seeds at the tested levels. Serum kidney functions and lipid profile were appreciably (P<0.05) decreased. At the same time, HDL-C was noticeably increased by psyllium seeds or husk supplementation at the tested levels in comparison to the positive control group.

***Keywords*---**hypocholesteremia, lipid profile, psyllium seed husk, psyllium seed, rats.

***Corresponding author**: Dhaher, A. G. A. E.; Email: ayatallahgalal@outlook.com*
Manuscript submitted: 27 Nov 2021, Manuscript revised: 18 Feb 2022, Accepted for publication: 09 March 2022

Introduction

Hypercholesterolemia causes cardiovascular disease and accounts for one-third of all mortalities globally. Synthetic hypercholesterolemic medicines have increasingly declined in popularity as a result of their associated adverse effects and the emergence of treatment resistance. So, the use of medicinal herbs has risen (Jørgensen et al., 2013). The American Heart Association defines hypercholesterolemia as a total blood cholesterol content of 240 mg/dl or above. It is a significant health condition that affects people all over the world. About 13 % of people aged 20 and above in the United States had elevated total cholesterol (Soslowsky & Fryhofer, 2016). Psyllium is the commonly used term for various plant species in the Plantago genus (Verma & Morga, 2013). Psyllium is a natural reservoir of lipids, alkaloids, and terpenoids Samuelsen (2000), and is high in polyphenols and flavonoids that act as antioxidants (Talukder et al., 2015).

Diets low in saturated fat and cholesterol, as well as diets containing seven grams or more of soluble psyllium seed husk fiber per day, have been shown to lower the risk of cardiovascular atherosclerosis (Shanahan et al., 2019). Jovanovski et al. (2018), found that Psyllium fiber (10.2 g) improved lipid profile, potentially slowing the progression of atherosclerosis-related CVD risk among persons with and without hypercholesterolemia. The findings suggested that psyllium seeds (0.5 and 1 %) had a robust cardioprotective effect and significant antioxidant activity. They protected tissue from oxidative stress due to their high content of dietary fibers and polyunsaturated fatty acids, flavonoids, and certain polyphenols (Ali, 2017). Therefore, this research aimed to determine the outcome of psyllium seeds and their husk on the lipid profile of hypercholesterolemic rats.

Material and Method

Material

Chemicals: The reagents kits for blood analysis were purchased from "Gama Trade Company for Chemical, Cairo, Egypt". bile salt, cellulose, minerals, vitamins, Casein, and cholesterol were purchased from "El-Gomhoria company – Cairo – Egypt". Herbs: Psyllium seeds and their husk were obtained from Agriculture Research Centre. Animals: Thirty-six adult male Albino rats were purchased from Agriculture Research Centre.

Methods

- Experimental animal and diet
  The animals were kept in good circumstances in "The Post Graduated Lab of the Faculty of Home Economics, Helwan University". They were kept in standard cages at room temperature (25 ± 3 °C) with a 12 h dark/ light cycle. They were given a basic diet Reeves et al. (1993), for seven days as an acclimatization period.
- Induction of hypercholesterolemic rats
  The hypercholesterolemic diet was prepared according to (Reeves et al., 1993) with some modifications containing Casein 14%, cellulose 5%, vitamin mixture 1%, sucrose 10%, mineral mixture 3.5%, choline bitartrate

0.25 %, corn oil 4%, l-cystine 0.18 and the remainder was starch plus cholesterol (1%) and bile salt (0.25%) to induce hypercholesterolemia in rats (Pandya et al., 2006).

After a period of acclimation, the rats were categorized into two groups: the first main group (n=6) was fed a standard diet as a normal control group (- ve) during the experimental period, and the second main group (n=30) was fed a high cholesterol diet. The rats were then split into 5 Subcategories, each with six rats: Subcategories (1): Rats were fed on high cholesterol diet (as a positive control group). Subcategories (2 and 3) were fed on high cholesterol diet and supplemented with 2.5 and 5% Psyllium husk powder, respectively. Subcategories (4 and 5) were kept on a high cholesterol diet and added 2.5 or 5 % Psyllium husk powder, respectively. Rats were fed for 12 hours before being slaughtered, and blood samples were taken and processed to extract serum, which was stored frozen at (-20˚c) until biochemical analysis. The determination of feed intake (FI) was used to conduct biological analyses. The feed efficiency ratio (FER) and body weight gain percent (BWG) were estimated using the equation of (Chapman et al., 1959).

Biochemical analysis

Serum total cholesterol (TC), triglycerides (TG), and HDL-c were determined according to Allen (1974); Fassati & Prencipe (1982); Lopez (1977) respectively. At the same time, LDL-c and very-low-density lipoprotein (VLDL-c) were determined according to the equation (Friedewable et al., 1972). According to Reitman & Frankel (1975), serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured (1957). According to Belfield & Goldberg (1971), serum alkaline phosphates (ALP) were measured (1971). Pattn and Crouch were used to determine serum urea and uric acid (1977), whereas Henry et al. (1974), was used to determine the serum creatinine (1974).

Statistical analysis

The obtained data were analyzed using the statistical analysis “program SPSS version, 20.0”. The grouped data was presented as “mean ± standard error (SE)”. Using the “ANOVA test” to determine the statistical significance of the difference among the different groups. The “$P < 0.05$” was considered statistically significant (Bailey, 1995).

Results and Discussion

The effect of psyllium seeds and their husk on the body weight status was illustrated in Table (1). There was no substantial difference in the initial body weight of all treated rats, while the final body weight (FBW), BWG%, and FER were significantly ($P<0.05$) decreased by Psyllium seeds or its husk at both levels compared to the +ve control group. There is a meaningful difference in FBW, BWG%, and FER among the treated groups. Psyllium husk was more effective in weight reduction than psyllium seeds; moreover, the higher percentage of either psyllium seeds or husk, the lower the weight reduction was observed. The most inferior reduction of FBW and BWG% was recorded at the group fed on psyllium husk at 5%, followed by psyllium seeds at 2.5%. The percent of weight reduction ranged between (-33 to -24%) for psyllium husk and its seeds, respectively.

The results mentioned above agreed with Jung et al. (2016); Ismael (2017); Mostafa (2019), who reported that BWG was significantly decreased (p<0.05) in dietary psyllium husk groups in comparison to the control group. Psyllium seeds or husk lowered the FI in the groups that consumed Psyllium seeds; this result agreed with Galisteo et al. (2005), who reported prolonged feeding with 3.5% psyllium husk decreased the BWG in obese rats. Giacosa & Rondanelli (2010), also reported that psyllium seed husk reduces body weight. Generally, weight loss by psyllium husk is believed to be associated with reduced plasma lipid concentrations (Jung et al., 2016).

Table 1
Effect of psyllium seeds and its husk on the body weight status of hypercholesterolemic rats

Parameters / Groups	IBW (g)	FBW(g)	BWG%	% of weight reduction	FI (g/d/rat)	FER
Control (-ve)	157.67±1.31[b]	179.50 ±1.13[b]	13.89±1.54[b]		15.40	0.032±0.03[b]
Control (+ve)	198.33±1.54[a]	235.03 ±0.91[a]	18.52±0.81[a]	30.93593	18.50	0.044±0.01[a]
Psyllium husk 2.5%	197.74±0.76[a]	161.00 ±0.81[d]	-18.73±0.13[d]	-31.4981	14.20	-0.058±0.002[e]
Psyllium husk 5%	200.00±1.08[a]	156.66 ±1.43[e]	-21.65±0.87[e]	-33.3447	13.70	-0.070±0.03[f]
Psyllium seed 2.5%	201.66±1.02[a]	177.83 ±0.77[b]	-11.80±0.82[b]	-24.3373	15.80	-0.034±0.02[c]
Psyllium seed 5%	200.65±1.31[a]	168.93 ±1.11[c]	-15.80±0.51[c]	-28.1241	16.00	-0.044±0.01[d]

The data is presented as a mean ± SE.
Values with unlike letters vs. other letters at P<0.05

The outcome of psyllium seeds and their husk on lipid profile was shown in Table (2). Psyllium seeds or husk at the tested levels appreciably (P<0.05) reduced the lipid parameters (TC, TG, VLDL-c, and LDL-c) and significantly increased serum HDL-c in comparison to the +ve control group. There is a substantial reduction in the mean values of (TC, TG, VLDL-C, and LDL-C) and a substantial increase in serum HDL-C for rats fed psyllium seeds at 5% compared with psyllium seeds 2.5%. The same trend was observed in the rats fed on psyllium husk 5% and 2.5%. The most remarkable improvement of lipid profile was recorded at the group fed on psyllium seeds 5%.

The hypolipidemic activity of psyllium seeds and husks due to soluble fiber, phenolic substance, flavonoids, oleic, linoleic, linolenic, caffeic, and chlorogenic acids contents. Flavonoids may function by raising the density of LDL-c receptors in the liver and binding to apo-lipoprotein B, allowing liver cells to remove LDL-c more efficiently from the bloodstream (Gunness & Gidley, 2010). Pourbehi et al. (2016), reported that there was a substantial decrease in TC, TG, and serum LDL levels and a significant increase in HDL levels in rats treated with psyllium seeds (5 g in 250 mL of water) or their extracts. Likewise, Ali (2017), found that supplementation with psyllium seeds at (0.5 and 1.0%) caused a decrease (p<0.05) in serum lipid profile levels and substantial boost (p<0.05) in serum HDL-c levels.

The cholesterol-lowering effect of psyllium husk refers to its ability to bind with the bile acids in the small intestine and hence decrease its absorption Xing et al. (2017), because of psyllium's physicochemical characteristics and the rise in meal viscosity (Galisteo et al., 2005). Other processes, such as propionate's inhibition of hepatic cholesterol synthesis and the indirect effects of decreasing glucose absorption, may also be involved (Madgulkar et al., 2015). It was reported that the significant increase in HDL-C levels refers to the capability of psyllium seeds to accelerate the decline of radical species created following cholesterol delivery. HDL-c reduces beta-lipoprotein peroxidation by acting as a free radical scavenger (Ismael & Shehata, 2020). Diabetic rats given psyllium husks at a concentration of 5% had the best levels of HDL and LDL cholesterol (Elhassaneen et al., 2021).

Another explanation of the cholesterol-lowering effect of psyllium seed husk fibers is decreased glycemic response due to reduced glucose absorption, linked to reduced insulin levels. Reduced insulin levels can inhibit “3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase)”, resulting in a decrease in hepatic cholesterol production (Ali, 2017). Generally, psyllium fiber supplementation was found to affect the lipid profile substantially and recommended for patients with a wide range of cardiovascular risk factors (Cicero et al., 2010). Moreover, psyllium reduces total cholesterol by 2% to 20% and LDL-c by 6% to 24% compared to placebo (McRorie, 2015).

Table 2
Effect of psyllium seeds and its husk on lipid profile of hypercholesterolemic rats

Parameters / Groups	TC	TG	VLDL-C (mg/dl)	HDL-C	LDL-C
Control (-ve)	108.00±1.08[f]	89.43±1.82[f]	17.88±0.36[f]	64.30±1.64[a]	25.81±1.92[f]
Control (+ve)	261.90±1.29[a]	160.03±1.12[a]	32.00±0.22[a]	34.43±0.89[e]	195.46±1.70[a]
Psyllium husk 2.5%	225.30±1.71[b]	147.16±1.35[b]	29.43±0.27[b]	42.13±0.98[d]	147.20±3.13[b]
Psyllium husk 5%	194.43±1.29[c]	131.30±1.43[c]	26.26±0.28[c]	48.66±1.69[c]	126.04±1.70[c]
Psyllium seed 2.5%	154.83±1.70[d]	120.03±1.53[d]	24.00±0.30[d]	56.96±1.78[b]	73.86±3.00[d]
Psyllium seed 5%	129.23±1.74[e]	107.40±1.02[e]	21.48±0.20[e]	61.50±1.13[a]	46.25±1.58[e]

The data is presented as a mean ± SE.
Values with unlike letters vs. other letters at P<0.05

The outcome of Psyllium seeds and their husk on serum liver functions were shown in Table (3). The positive control group had a substantial (P<0.05) increase in AST, ALT, and ALP levels in comparison to the negative control group. The supplemented Psyllium husk or seed at the different levels significantly (P<0.05) decreased the mean levels of liver functions in comparison to the +ve control group. Besides, there are substantial alterations among the treated groups. It was also observed that the higher percentage of Psyllium seed or husk supplementation, the lower liver enzymes.

These findings might be due to the components of psyllium husk “9,12-octadecadienoic acid, methyl ester, and -sitosterol,” which act as hepatoprotective agents (Devaraj et al., 2020). Likewise, Elhardallou et al. (2015); Ali (2017), found that diabetic rats fed a diet enriched with psyllium seed husk, alone or in combination, for 4 weeks discovered a significant reduction in liver enzymes in comparison to the positive control group. The obtained findings were also in line with Hashem et al. (2021), who found that psyllium husk ethanolic extract (250 g was extracted in 1L 70% ethanol) significantly reduced liver parameters in triton-induced hyperlipidemic rats.

Table 3
Effect of psyllium seeds and its husk on serum liver functions of hypercholesterolemic rats

Parameters / Groups	AST	ALT (μ/L)	ALP
Control (-ve)	70.03±0.38[f]	23.36±0.60[f]	60.33±1.24[f]
Control (+ve)	121.53±1.08[a]	45.76±0.76[a]	115.06±1.08[a]
Psyllium husk 2.5%	84.76±0.83[d]	31.93±1.43[d]	82.01±2.24[d]
Psyllium husk 5%	76.53±0.57[e]	27.56±0.90[e]	74.18±1.12[e]
Psyllium seed 2.5%	105.83±1.85[b]	39.73±0.66[b]	94.27±0.96[b]
Psyllium seed 5%	90.40±1.98[c]	36.86±0.85[c]	89.20±1.93[c]

The data is presented as a mean ± SE.
Values with unlike letters vs. other letters at P<0.05

Table (4) showed the outcome of Psyllium seeds and their husk on serum kidney functions of hypercholesterolemic rats. Serum urea, uric acid, and creatinine are significantly (P<0.05) increased for the +ve control group in comparison to the (-ve) control group. While the supplementation with different levels of psyllium seeds or husk significantly decreased the level of kidney functions in contrast to the (+ve) control group. There are no substantial serum urea or uric acid changes among Psyllium husk 2.5% and Psyllium husk 5%. However, Psyllium seed at 5% significantly decreased the serum urea and uric acid compared to Psyllium husk 2.5%. There is no significant difference between the rats fed either Psyllium seed 2.5 or 5% regarding serum creatinine. The most remarkable improvement in kidney functions was seen in the rats fed on Psyllium husk 5% in comparison to the other regimens.

Similarly, de Bock et al. (2012), found that psyllium husk supplementation with 6 g/day over 6 weeks, serum urea and creatinine levels were significantly lower (P>0.01) than in the control group. Elhardallou et al. (2015), also reported that a diet enriched with psyllium husk lowered serum urea levels among rats. Elhassaneen et al. (2021), reported that rats treated with psyllium husks at 5% significantly decreased values of urea in comparison to psyllium seeds, while no significant differences between them were shown for uric acid and creatinine.

Table 4
Effect of psyllium seeds and its husk on serum kidney function of hypercholesterolemic rats

Parameters / Groups	Urea	Uric acid (mg/dl)	Creatinine
Control (-ve)	40.65±0.83[d]	0.983±0.01[d]	0.371±0.06[d]
Control (+ve)	79.05±1.82[a]	1.86±0.04[a]	1.01±0.01[a]
Psyllium husk 2.5%	70.28±1.34[b]	1.68±0.03[b]	0.924±0.02[b]
Psyllium husk 5%	67.19±0.99[b]	1.65±0.02[b]	0.799±0.02[c]
Psyllium seed 2.5%	71.82±1.200[b]	1.67±0.04[b]	0.786±0.02[c]
Psyllium seed 5%	59.00±2.51[c]	1.54±0.03[c]	0.754±0.01[c]

The data is presented as a mean ± SE.
Values with unlike letters vs. other letters at $P<0.05$

Finally, it could be concluded that Psyllium seeds at (5%) had a significant hypocholesterolemic effect and improved kidney and liver functions. So, Psyllium seeds may be recommended for hypercholesterolemic patients.

References

Ali, A. I. (2017). Hypoglycemic and hypolipidemic effects of dietary supplementation of plantago psyllium seeds on diabetic and/or hyperlipidemic albino rats. *Journal of Scientific Research in Science*, *34*(part1), 373-398.

Allain, C. C. (1974). Cholesterol enzymatic colorimetric method. *J. of Clin. Chem*, *20*, 470.

Bailey, N. T. (1995). *Statistical methods in biology*. Cambridge university press.

Belfield, A., & Goldberg, D. M. (1971). Alkaline phosphatase colorimetric method. J. of Enzyme,(12): 561.

Chapman, D. G., Castillo, R., & Campbell, J. A. (1959). Evaluation of protein in foods: 1. A method for the determination of protein efficiency ratios. *Canadian Journal of Biochemistry and Physiology*, *37*(5), 679-686.

Cicero, A. F., Derosa, G., Bove, M., Imola, F., Borghi, C., & Gaddi, A. V. (2010). Psyllium improves dyslipidaemia, hyperglycaemia and hypertension, while guar gum reduces body weight more rapidly in patients affected by metabolic syndrome following an AHA Step 2 diet. *Mediterranean Journal of Nutrition and Metabolism*, *3*(1), 47-54.

de Bock, M., Derraik, J. G., Brennan, C. M., Biggs, J. B., Smith, G. C., Cameron-Smith, D., ... & Cutfield, W. S. (2012). Psyllium supplementation in adolescents improves fat distribution & lipid profile: a randomized, participant-blinded, placebo-controlled, crossover trial.

Devaraj, E., Roy, A., Royapuram Veeraragavan, G., Magesh, A., Varikalam Sleeba, A., Arivarasu, L., & Marimuthu Parasuraman, B. (2020). β-Sitosterol attenuates carbon tetrachloride–induced oxidative stress and chronic liver injury in rats. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *393*(6), 1067-1075.

Elhardallou, S. B., Babiker, W. A., Sulieman, A. M. E., & Gobouri, A. A. (2015). Effect of Diet Supplementation with Food Industry By-Products on Diabetic Rats. *Food and Nutrition sciences*, *6*(10), 875.

Elhassaneen, Y., Rhman, A. N. A., & Hussin, N. A. (2021). The Potential Effects of Psyllium Seeds and its Husks (Plantago ovata) on Diabetic Rats.

Fassati, P., & Prencipe, L. (1982). Triglyceride enzymatic colorimetric method. *J. Clin. Chem*, *28*, 2077.

Friedewald, W. T., Levy, R. I., & Fredrickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical chemistry*, *18*(6), 499-502.

Galisteo, M., Sanchez, M., Vera, R., González, M., Anguera, A., Duarte, J., & Zarzuelo, A. (2005). A diet supplemented with husks of Plantago ovata reduces the development of endothelial dysfunction, hypertension, and obesity by affecting adiponectin and TNF-α in obese Zucker rats. *The Journal of nutrition*, *135*(10), 2399-2404.

Giacosa, A., & Rondanelli, M. (2010). The right fiber for the right disease: an update on the psyllium seed husk and the metabolic syndrome. *Journal of clinical gastroenterology*, *44*, S58-S60.

Gunness, P., & Gidley, M. J. (2010). Mechanisms underlying the cholesterol-lowering properties of soluble dietary fibre polysaccharides. *Food & function*, *1*(2), 149-155.

Hashem, M. A., Abd-Allah, N. A., Mahmoud, E. A., Amer, S. A., & Alkafafy, M. (2021). A Preliminary Study on the Effect of Psyllium Husk Ethanolic Extract on Hyperlipidemia, Hyperglycemia, and Oxidative Stress Induced by Triton X-100 Injection in Rats. *Biology*, *10*(4), 335.

Henry, R. J., Cannon, D. C., & Winkelman, J. W. (1974). Clinical Chemistry, Principles and Techniques. 2nd.(ed.) Harber and Row Company.

Ismael, N. M., & Shehata, M. G. (2020). Improvement of lipid profile and antioxidant of hyperlipidemic albino rats by functional Plantago psyllium Cake. *Current Research in Nutrition and Food Science Journal*, *8*(2), 424-437.

Ismael, S. (2017). Biological and histopathological effects of psyllium husk seed and dome in hypercholesterolemic rats. *African Journal of Biological Sciences*, *13*(1), 121-138.

Jørgensen, T., Capewell, S., Prescott, E., Allender, S., Sans, S., Zdrojewski, T., ... & Vanuzzo, D. (2013). Population-level changes to promote cardiovascular health. *European journal of preventive cardiology*, *20*(3), 409-421.

Jovanovski, E., Yashpal, S., Komishon, A., Zurbau, A., Blanco Mejia, S., Ho, H. V. T., ... & Vuksan, V. (2018). Effect of psyllium (Plantago ovata) fiber on LDL cholesterol and alternative lipid targets, non-HDL cholesterol and apolipoprotein B: a systematic review and meta-analysis of randomized controlled trials. *The American journal of clinical nutrition*, *108*(5), 922-932.

Jung, E. Y., Hong, Y. H., Chang, U. J., & Suh, H. J. (2016). Anti-obese effects of chitosan and psyllium husk containing vitamin C in Sprague-Dawley (SD) rats fed a high fat diet. *Progress in Nutrition*, *18*(2), 152-160.

Lopez, M. F. (1977). HDL-cholesterol colorimetric method. *J. of Clin. Chem*, *230*, 282.

Madgulkar, A. R., Rao, M. R., & Warrier, D. (2015). Characterization of psyllium (Plantago ovata) polysaccharide and its uses. *Polysaccharides*, 871-890.

McRorie Jr, J. W. (2015). Evidence-based approach to fiber supplements and clinically meaningful health benefits, part 1: what to look for and how to recommend an effective fiber therapy. *Nutrition today*, *50*(2), 82.

Mostafa, M. Y. (2019). Effect of Feeding Psyllium Husk (Plantago Ovata) Herbs on Rats suffering from Hyperglycemia. □□□□ □□□□□□□ □□□□□□ □□□□□□□□□ □□□□□□□□, *1*(2).

Pandya, N., Santani, D., & Jain, S. (2006). Antioxidant activity of ezetimibe in hypercholesterolemic rats. *Indian journal of pharmacology*, *38*(3), 205.

Pattn,C. and Crouch, S. (1977): Enzymatic determination of urea'.J.Anal.Chem.,49:464-469

Pourbehi, F., Ayremlou, P., Mehdizadeh, A., & Zarrin, R. (2016). Effect of Psyllium Supplementation on Insulin Resistance and Lipid Profile in Non-diabetic Women With Polycystic Ovary Syndrome: A Randomized Placebo-Controlled Trial. *Hospital*, *2017*.

Reeves, P. G., Nielsen, F. H., & Fahey Jr, G. C. (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet.

Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American journal of clinical pathology*, *28*(1), 56-63.

Samuelsen, A. B. (2000). The traditional uses, chemical constituents and biological activities of Plantago major L. A review. *Journal of ethnopharmacology*, *71*(1-2), 1-21.

Shanahan, C. J., Gibb, R. D., McRorie, J. W., Brum, J. M., & Ritchey, M. E. (2019). Economic impact analysis of the coronary heart disease-attributed health care cost savings and productivity gains from the use of Psyllium. *Nutrition & Food Science*.

Soslowsky, L. J., & Fryhofer, G. W. (2016). Tendon homeostasis in hypercholesterolemia. *Metabolic Influences on Risk for Tendon Disorders*, 151-165.

Talukder, P., Talapatra, S., Ghoshal, N., & Raychaudhuri, S. S. (2015). Antioxidant activity and HPLC analysis of phenolic compounds during in vitro callus culture of Plantago ovata Forsk and effect of exogenous additives on accumulation of phenolic compounds. *J. Sci. Food Agric*, *96*, 232-244.

Verma, A., & Mogra, R. (2013). Psyllium (Plantago ovata) husk: a wonder food for good health. *International Journal of Science and Research*, *4*(9), 1581-1585.

Xing, L. C., Santhi, D., Shar, A. G., Saeed, M., Arain, M. A., Shar, A. H., ... & Ling, M. C. (2017). Psyllium husk (Plantago ovata) as a potent hypocholesterolemic agent in animal, human and poultry. *International Journal of Pharmacology*, *13*(7), 690-697.