ORIGINAL ARTICLE

Impact of Aqueous Extract of Plantago Ovata Fork (Psyllium) Fruit on the Improvement of Non-alcoholic Fatty Liver Disease among Male Rats

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Abstract

Background and Aim: Non-alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of disorder and damage to the liver. *Plantago psyllium* possesses hypolipidemic (fat reducing) and antioxidant properties. According to increased prevalence of NAFLD and the positive impact of some herbal remedies (antioxidants) in the prevention and treatment of disease, this study was aimed to investigate the effect of psyllium extract on NAFLD. **Materials and Methods:** In this experimental study, 56 adult male Wister rats were divided into seven groups of eight animals including control and sham 1 and 2 (induced fatty liver). Experimental group 1 received psyllium extract with a concentration of 400 mg/kg and experimental groups 2, 3, and 4 received psyllium extract with a concentration of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively, after the induction of fatty liver over 28 consecutive days. Blood samples were taken from the animal's heart a day after the last injection, and alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) liver enzymes concentration as well as low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol (TC) were measured. The results were analyzed through analysis of variance and Duncan's test. The statistical significance level was set at $P > 0.05$. **Findings:** The mean serum HDL, TC, and triglycerides (TG) levels were significantly decreased in the sham groups 1 and 2 compared to the control group. The mean serum ALT, ALP, TC, and TG levels were significantly decreased in the experimental group 1 compared to the control group. The mean serum ALT, AST, and ALP levels were significantly decreased in the experimental groups 3 and 4 compared to the sham groups 1 and 2, but the mean serum ALT and ALP levels in the experimental group 2 were significantly different from the sham groups 1 and 2, and no change was found in the mean serum AST level. Furthermore, in the experimental group 3, the mean serum TC and TG levels were decreased significantly compared to the sham group 1 and the control group, and the mean serum TG also showed a significant decrease in the experimental group 3 compared to the sham group 2. However, there was no change in the mean serum TC in the experimental group 3 compared to the sham group 2. However, there was no change in the mean serum TC in the experimental group 3 compared to the sham group 2. The mean serum HDL level showed a significant increase in the experimental group 4 compared with the sham group 2 and decreased significantly in the experimental groups 2, 3, and 4 compared to the control group. The mean serum LDL level was significantly decreased in experimental (2, 3, and 4) groups compared to the sham groups (1 and 2), but there was no change compared to the control group. **Conclusion:** According to the results, the psyllium extract can probably induce hepatoprotective effect and improvement of liver enzymes disorder as well as lipid profile in non-alcoholic fatty liver patients due to the antioxidant properties.

Key words: Non-alcoholic fatty liver, plantago ovata fork (psyllium) fruit, rat

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) was firstly detected by Ludwig *et al.* (1980) in people with no history of alcohol intake.[1] NAFLD encompasses the simple steatosis to more progressive steatosis with associated hepatitis, fibrosis, and cirrhosis.[2] NAFLD is now considered the hepatic manifestation of metabolic syndrome and its clinical manifestations include type-2 diabetes, obesity, dyslipidemia, and hypertension. The prevalence of NAFLD in the general population in western countries is 20–30% and due to the rapidly increasing prevalence of risk factors of metabolic syndrome, it is now considered as the most common liver disease in the western world.[3] Epidemiological studies reported the prevalence of NAFLD as 2.8% in Iranian population.[4] The pathogenesis of NAFLD is not fully understood. Current evidence suggests that insulin resistance, obesity, oxidative stress, and inflammatory cascade play a role in the pathogenesis and progression of the disease.[5] The "two-hit" hypothesis has been elaborated to explain the pathogenesis of NAFLD which was firstly proposed by Day and James. The first hit, insulin resistance and metabolic disturbances, results in hepatic lipid accumulation and sensitizes the liver to a variety of "second hits" including potential sources of oxidative stress.[6] Oxidative stress can contribute to increased lipid peroxidation in the liver cell plasma membrane. Oxidative by-products are hazardous and can influence nucleotide and protein synthesis. These compounds can also result in an increased secretion of inflammatory cytokines and activation of hepatic stellate cells, and consequently, fibrosis, inflammation, and apoptosis.[7,8] No exclusive treatment has not yet been detected for these patients (the treatment for fatty liver disease is dependent on treating other risk factors such as diabetes or high cholesterol level). However, due to the role of oxidative stress in the pathogenesis of the disease and low levels of antioxidants in these patients, many researchers have focused a great deal of attention on the use of antioxidants in the treatment of the disease.[9] Antioxidants contain a mix of nutrients including vitamins and minerals found in foods. The main antioxidants in foods including beta-carotene (a precursor of Vitamin A presents in dark orange fruits and vegetables), Vitamin C, which can be found in fruits, especially citrus fruits, Vitamin E in vegetable oils (such as olive oil, canola), kernels such as walnuts and almonds, and some minerals and herbs possess antioxidant properties like *Plantago psyllium*.[10] The genus Plantago belongs to the family Plantaginaceae and comprises about 250 species. Psyllium has a cosmopolitan distribution and is cultivated primarily in India and Pakistan.[11] Plantago ovata fork and *P. psyllium* L., which is known as psyllium in Iran, are widely used in pharmaceutical industries.[12] Psyllium contains compounds such as oleic acid, luteolin, linoleic acid, *beta-sitosterol*, fructose, glucose, lignoceric acid and *acubine* and campesterol, sucrose, alpha-amyrin, palmitic acid, and phenolic compounds; among which *acubin*e, luteolin, campesterol, and sucrose have antioxidant properties.[13] In a study by *Stankovi et al*. on the correlation between fatty acids released from the liver and serum lipid levels as well as histological features in fatty liver of non-alcoholic mice, it was concluded that total cholesterol, high-density lipoprotein (HDL), and triglyceride decreased gradually, whereas a gradual increase occurred in low-density lipoprotein (LDL) level.[14] Findings of the study by Aijaz Ahmed *et al.* showed that fatty liver is the most common cause of elevated serum aminotransferase levels.[15] In a study by Lerman-Garber *et al*. on the effect of psyllium *plantago* in moderate-to-severe hypercholesterolemic patients, it was concluded that *P. psyllium* produced a reduction of 8% in total cholesterol and 11% in LDL cholesterol after 12 weeks, but no change was observed in triglycerides and *HDL*-cholesterol levels.[16] Therefore, the aim of this study was to investigate the therapeutic effect of plantago ovata fork (psyllium) fruit on the improvement of non-alcoholic fatty liver in adult rats.

MATERIALS AND METHODS

In this experimental study, 56 male Wistar rats weighing between 150 and 180 g were recruited. The rats were housed for a week in the animal breeding room of Jahrom University of Medical Sciences to adapt to the environment. They were exposed to either a normal cycle of 12 h of light and 12 h of darkness and the humidity was around 50–55%. All ethical principles regarding the use of laboratory animals were also taken into consideration. The inclusion criteria included healthy male Wistar rats weighing between 150–180 g and exclusion criteria were death of animals during the course of experimentation. Samples were selected using convenient sampling method and divided into groups using a random number table. In accordance with the previous articles, the psyllium extract was administered at doses of 100, 200, and 400 mg/kg body weight to groups.[17] Therefore, the control and experimental groups consisted of the following groups.

Control group

This group did not receive any treatment during the experiment ($n = 8$).

Control group 1

This group received high-fat diet for 4 consecutive weeks. Autopsies were performed immediately on animals and their tissues and serum were sent out to the laboratory to ensure that the fatty liver in the mice was induced ($n = 8$).

Control group 2

This group received high-fat diet for 4 consecutive weeks and then regular food for 28 days ($n = 8$).

Experimental group 1

This group received the aqueous extract of psyllium at a concentration of 400 mg/kg body weight by oral gavage for 28 days ($n = 8$).

Experimental group 2

This group received an aqueous extract of psyllium at a concentration of 100 mg/kg body weight by oral gavage for 28 days (after induction of fatty liver) ($n = 8$).

Experimental group 3

This group received an aqueous extract of psyllium fruits at a concentration of 200 mg/kg body weight by oral gavage for 28 days (after induction of fatty liver) ($n = 8$).

Experimental group 4

This group received an aqueous extract of psyllium at a concentration of 400 mg/kg body weight by oral gavage for 28 days (after induction of fatty liver) ($n = 8$). 5 cc blood was directly taken from the heart of animals using a syringe (on day 29 after complete induction of non-alcoholic fatty liver) under anesthesia by diethyl ether at the end of the study. They were centrifuged at 3000 rpm for 15 min and stored at −20°C for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglycerides (TG), total cholesterol (TC), HDL, and LDL measurement. Pars laboratory test kits were used to measure these parameters. One-way analysis of variance and Duncan test were used to analyze the data through SPSS software. The statistical significance level was set at 0.05 (5%).

RESULTS

The mean serum HDL, TC, and TG levels were significantly decreased in the sham groups 1 and 2 compared to the control group [Figures 1-3].

The mean serum ALT, ALP, TC, and TG levels were significantly decreased in the experimental group 1 compared to the control group [Figures 1,2,4,5].

The mean serum ALT, AST, and ALP levels were significantly decreased in the experimental groups (3 and 4) compared to the sham groups 1 and 2, but the mean serum ALT and ALP levels in the experimental group 2 were significantly different from the sham groups 1 and 2, and no change was found in the mean serum AST level [Figures 4-6].

In the experimental group 3, the mean serum TC and TG levels were decreased significantly compared to the sham group 1 and the control group. The mean serum TG also showed a significant decrease in the experimental group 3 compared to the sham group 2. However, there was no change in the mean serum TC in the experimental group 3 compared to the sham group 2 [Figures 1 and 2].

The mean serum TC and TG levels showed a significant decrease compared to the sham groups 1 and 2 and the control group in the experimental group 4 [Figures 1 and 2].

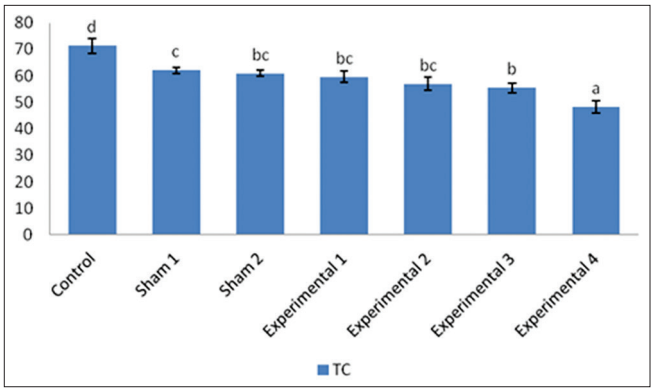

Figure 1: Average serum total cholesterol concentration in the studied groups. Means presented within each column with common letter are not significantly different according to Duncan test

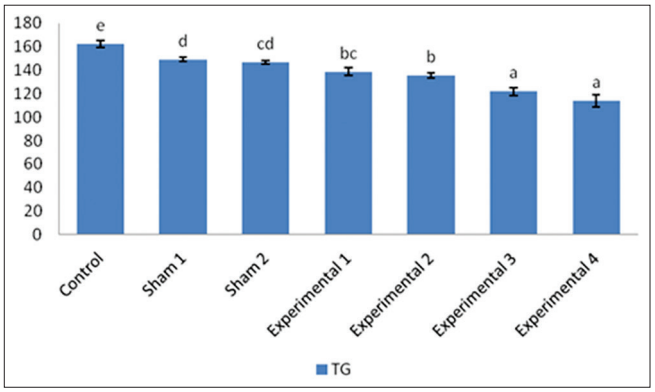

Figure 2: Average serum triglycerides concentration in the studied groups. Means presented within each column with common letter are not significantly different according to Duncan test

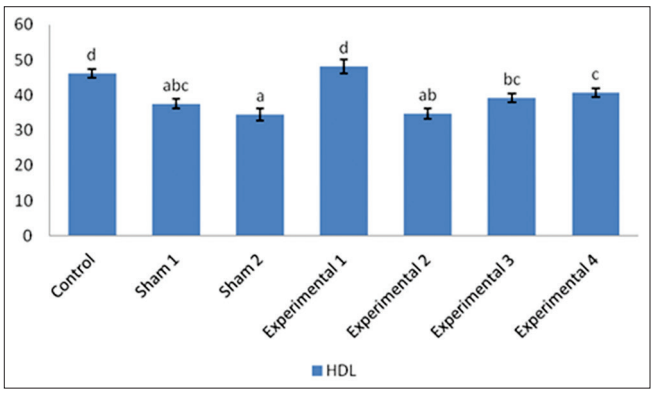

Figure 3: Average serum high-density lipoprotein concentration in the studied groups. Means presented within each column with common letter are not significantly different according to Duncan test

A significant decrease was observed in the mean serum TG level of the experimental group 2 compared to the sham groups 1 and 2, but no change was seen in the mean serum TC level [Figures 1 and 2].

The experimental group 4 showed a significant increase in the mean serum HDL level compared with the sham group 2 and decreased significantly in the experimental groups (2, 3, and 4) compared to the control group [Figure 3].

The mean serum LDL level was significantly decreased in experimental groups (2, 3, and 4) compared to the sham groups (1 and 2), but no change was found compared to the control group [Figure 7].

Comparison of the results of experimental groups receiving different doses revealed that the aqueous extract of psyllium at a concentration of 400 mg/kg plays an effective role in decreasing serum levels of ALT, AST, ALP, TC, TG, and LDL and increasing serum HDL compared to other doses.

DISCUSSION

NAFLD is characterized by excessive accumulation of fat in the liver of people who drink little or no alcohol. This disease has become increasingly recognized as a worldwide problem and treatment of patients with the disease is becoming frequent problem in clinical practice. Since then, herbal remedies made a significant contribution to the treatment of these patients. They can play an important role in preventing and treating the disease with minimal side effects and maximum efficacy. The results of this study showed that the aqueous extract of psyllium could improve the serum concentration of liver enzymes and lipid profiles in male rats due to its strong antioxidant effect, and it has also been shown that a dose of 400 mg/kg is the most effective and recommended concentration for treatment. In other words, this aqueous extract at a concentration of 400 mg/kg has an effective role in reducing the serum levels of ALT, AST, and ALP compared to other doses. The findings of a study by Akbarzadeh *et al.* (2017) suggested that the use of PP followed by dietary supplements in people with overweight and non-alcoholic fatty liver for 10 weeks could lead to a significant reduction in body weight, waist circumference, body fat percentage, and plasma levels of ALT compared with placebo group.[18] Moreover, other studies have found that physical activity and diet can lead to a significant decrease in weight, body mass index, and ALT plasma level.[19] According to the results of other studies, metabolic syndrome treatment is one of the main recommendations for treating patients with non-alcoholic fatty liver. Some healthy diets including unsaturated fats and plenty of vegetables and fruits are useful for patients with non-alcoholic fatty liver.[20] Exposure to excess body fat, especially intra-abdominal fat in obese people with non-alcoholic steatohepatitis, is associated with increased risk of metabolic complications.[21,22] In addition, it should be noted that high level of free fatty acids released and decreased beta-oxidation in the liver induced by active oxygen species can cause additional oxidative stress and destructive inflammatory responses.[20] Based on the results of this study, the aqueous extract of psyllium at the dose of 400 mg/kg can significantly reduce serum levels of TC, TG, and LDL compared to other doses and increase serum HDL level; thus, the body fat percentage is found to decrease. The findings from other study also suggest that high doses of psyllium extract can reduce body weight, body mass index,

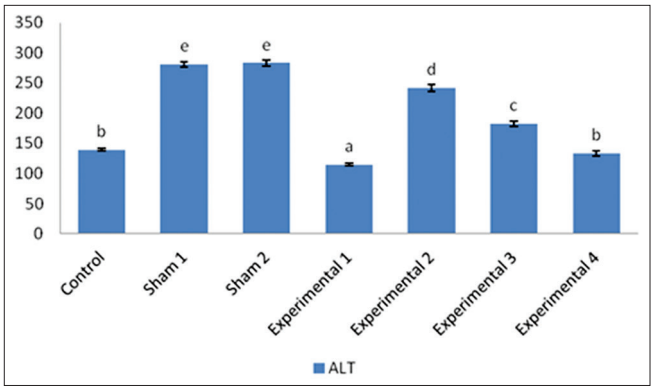

Figure 4: Average serum alanine aminotransferase concentration in the studied groups. Means presented within each column with common letter are not significantly different according to Duncan test

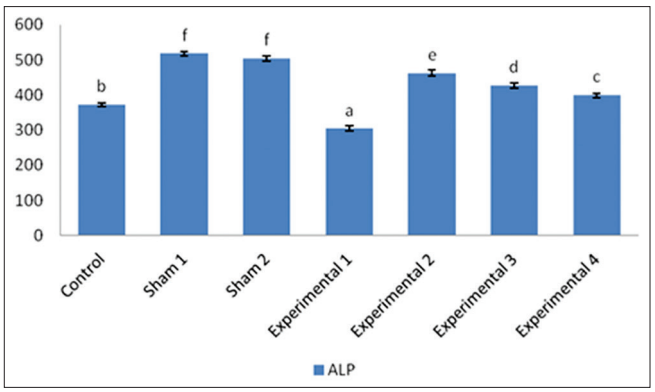

Figure 5: Average serum alkaline phosphatase concentration in the studied groups. Means presented within each column with common letter are not significantly different according to Duncan test

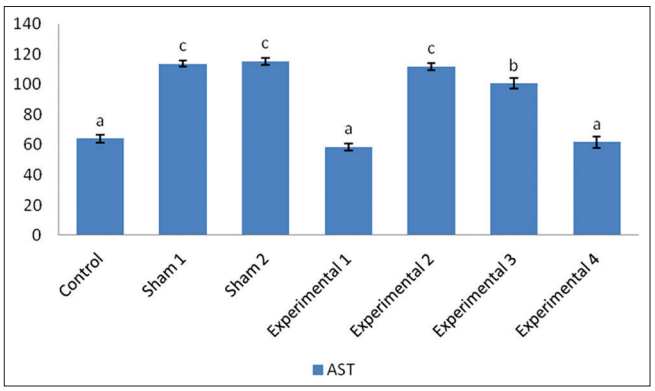

Figure 6: Average serum aspartate aminotransferase concentration in the studied groups. Means presented within each column with common letter are not significantly different according to Duncan test

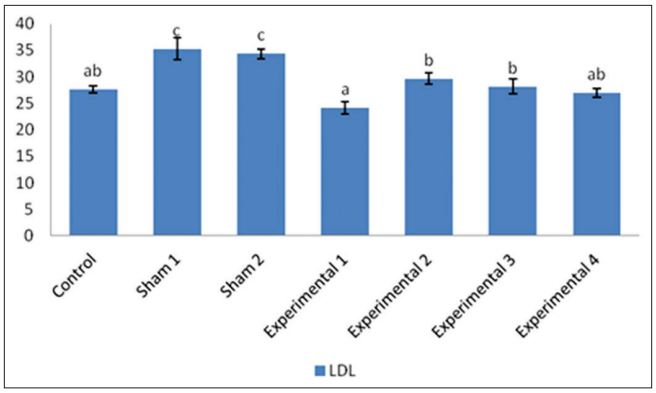

Figure 7: Average serum low-density lipoprotein concentration in the studied groups. Means presented within each column with common letter are not significantly different according to Duncan test

and total body fat percentage.[23] It was also reported that psyllium can make cells sensitive to insulin through reducing the free fatty acids and lowered release of glycerol from adipose tissue. Therefore, psyllium is considered to be an herbal remedy for delaying the liver damages or prevention in patients with unsaturated fatty liver.

CONCLUSION

According to the results, the psyllium extract can probably induce hepatoprotective effect and improvement of liver enzymes disorder as well as lipid profile in non-alcoholic fatty liver patients due to the antioxidant properties.

REFERENCES

1. McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. Clin Liver Dis 2004;8:521-33, viii.
2. Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, *et al.* Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917-23.
3. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S, *et al.* Prevalence of and risk factors for nonalcoholic fatty liver disease: The dionysos nutrition and liver study. Hepatology 2005;42:44-52.
4. Jamali R, Khonsari M, Merat S, Khoshnia M, Jafari E, Bahram Kalhori A, *et al.* Persistent alanine aminotransferase elevation among the general iranian population: Prevalence and causes. World J Gastroenterol 2008;14:2867-71.
5. Jou J, Choi SS, Diehl AM.Mechanisms of disease progression in nonalcoholic fatty liver disease. Semin Liver Dis. 2008;28:370-9.
6. Day CP, James OF. Steatohepatitis: A tale of two "hits"? Gastroenterology 1998;14:2867.
7. Edmison J, McCullough AJ. Pathogenesis of non-alcoholic steatohepatitis: Human data. Clin Liver Dis 2007;11:75-104, ix.
8. Madan K, Bhardwaj P, Thareja S, Gupta SD, Saraya A. Oxidant stress and antioxidant status among patients with nonalcoholic fatty liver disease (NAFLD). J Clin Gastroenterol 2006;40:930-5.
9. Younossi ZM. Review article: Current management of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Aliment Pharmacol Ther 2008;28:2-12.
10. Rice-Evans CA, Miller NJ. Antioxidant activities of flavonoids as bioactive components of food. Biochem Soc Trans 1996;24:790-5.
11. Chakrabort MK, Patel KV. Chemical-composition of Isabgol (*Plantago ovata* forsk) seed. J Food Sci 1992;8:389-90.
12. Ghahraman A. Fluorescent of Iran. Vol. 6. Tehran: Research institute of Forest and Range; 1984.
13. Pandita D. Cytomeiotic analysis of Plantago ovata forsk–an indian restorative plant. J Plant Animal Environ Sci 2013;3(3):118-121.
14. Stanković MN, Mladenović DR, Duričić I, Šobajić SS, Timić J, Jorgačević B, *et al.* Time-dependent changes and association between liver free fatty acids, serum lipid profile and histological features in mice model of nonalcoholic fatty liver disease. Arch Med Res 2014;45:116-24.
15. Ahmed A, Wong RJ, Harrison SA. Nonalcoholic fatty liver disease review: Diagnosis, treatment, and outcomes. Clin Gastroenterol Hepatol 2015;13:2062-70.
16. Lerman Garber I, Lagunas M, Sienra Pérez JC, Ahumada Ayala M, Saldaña A, Cardoso Saldaña G, *et al.* The effect of psyllium *Plantago* in slightly to moderately hypercholesterolemic patients. Arch Inst Cardiol Mex 1990;60:535-9.
17. Lee J, Chae K, Ha J, Park BY, Lee HS, Jeong S, *et al*. Regulation of obesity and lipid disorders by herbal extracts from *Morus alba*, *Melissa officinalis*, and *Artemisia capillaris* in high-fat diet-induced obese mice. J Ethnopharmacol 2008;115:263-70.
18. Nourian M, Akbarzadeh Z, Askari G, Maracy MR. The effect of psyllium on body composition measurements and liver enzymes in overweight or obese adults with nonalcoholic. IJBR 2016;7:1545-54.
19. Catalano D, Trovato GM, Martines GF, Randazzo M, Tonzuso A. Bright liver, body composition and insulin resistance changes with nutritional intervention: A follow-up study. Liver Int 2008;28:1280-7.
20. Oliveira LP, de Jesús RP, Freire TO, Oliveira CP, Castro Lyra A, Lyra LG, *et al.* Possible molecular mechanisms soy-mediated in preventing and treating nonalcoholic fatty liver disease. Nutr Hosp 2012;27:991-8.
21. Gutierrez-Grobe Y, Gavilanes-Espinar JG, Mendez-Sanchez N. Physical activity and nonalcoholic fatty liver disease. Ann Hepatol 2009;8:273-4.
22. Schwimmer JB, Pardee PE, Lavine JE, Blumkin AK, Cook S. Cardiovascular risk factors and the metabolic

syndrome in pediatric nonalcoholic fatty liver disease. Circulation 2008;118:277-83.
23. Pal S, Khossousi A, Binns C, Dhaliwal S, Ellis V. The effect of a fibre supplement compared to a healthy diet on body composition, lipids, glucose, insulin and other metabolic syndrome risk factors in overweight and obese individuals. Br J Nutr 2011;105:90-100.

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