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The Potential Effects of Psyllium Seeds and its Husks (*Plantago ovata*) on Diabetic Rats

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Abstract:

This study was carried out to investigate the potential effects of psyllium seeds and its husks on diabetic rats. Thirty- six male albino rats (170 ± g) were divided in two main groups. The first, negative control group (n = 6), fed on basal diet and the second group (diabetic rats, n = 30). Second group was divided into 5 subgroups (6 rats each). First subgroup is positive control fed on basal diet, the second and third subgroups fed on basal diet supplemented with 3 and 5% psyllium seeds respectively and fourth and fifth subgroups fed on basal diet supplemented with 3 and 5% psyllium husks respectively. At the end of the experimental period (30 days), animals were scarified for blood collection. Serum glucose, 1insulin, glycated hemoglobin (HbA1C), lipids profile, liver and kidney functions were determined. Histological examination for liver was done. Feeding diabetic rats with diet supplemented with psyllium seeds or husks significantly decreased the levels of blood glucose, triglyceride, very low-density lipoprotein cholesterol and liver functions. However, there was no significant difference ($P \leq 0.5$) in insulin, HbA1C, low density lipoprotein cholesterol, high-density lipoprotein and kidney functions among positive control group and groups treated with 3 and 5 % of psyllium seeds and husks. Supplementation rat diets with psyllium husks (5%) were more effective than psyllium husks (3%) and psyllium seeds (3 and 5%) in improvement the histological of liver. Increasing the doses could become more apparent.

Keywords: psyllium, insulin, liver functions, glycated hemoglobin

Introduction

Diabetes is a serious, long-term condition with a major impact on the lives and well-being of individuals, families, and societies worldwide. It is predicted that 578 million people will have diabetes in 2030 and the number will increase by 51% (700 million) in 2045

(IDF 2019). Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term tissue and organ damage, as well as dysfunction involving the eyes, kidneys, nerves, heart, and blood vessels (ADA, 2014).The presence of chronic hyperglycemia in DM is accompanied by greater or lesser impairment in the metabolism of carbohydrates, lipids and proteins. Also, hyperlipidemia is the most common complication of diabetes mellitus and it predisposes them to premature atherosclerosis and macrovascular complications. (Uttra et al., 2011). Nutrition, exercise and lifestyle modifications are among the key approaches for the management and prevention of diabetes (Hordern et al., 2012); (Balk et al., 2015) and (Razaz et al., 2019). Psyllium, which is a water-soluble fiber derived from the husks of ripe seeds (Abutair et al., 2016). Psyllium plant parts (leaves, seeds, and husks) are rich in bioactive compounds and different primary and secondary metabolites (Talukder et al., 2015). The dietary fibers from psyllium have been used extensively both as pharmacological supplements and food ingredients in processed food to aid weight control, to regulation of glucose control for diabetic patients and to reducing serum lipid levels in hyperlipidemic. The pharmacological importance of psyllium polysaccharide and therapeutic value of psyllium for the treatment of constipation was diarrhea, irritable bowel syndrome, inflammatory bowel disease-ulcerative colitis, colon cancer, diabetes and hypercholesterolemia and exploitation of psyllium for developing drug delivery system (Singh, 2007). They are a dearth information about the relationship between Psyllium consumption and diabetes mellitus. Therefore, the purpose of this study was to investigate the potential effects of psyllium husks and its seeds on diabetic rats.

Materials and methods

Materials

Psyllium husks and seeds were obtained from Harraz Co. for Agricultural Seeds, Spices and Medicinal plants, Cairo, Egypt. Alloxan and kits were obtained from El-Gomhoria Company for Trading Drug Chemicals and medicals, Cairo, Egypt. All other chemicals and reagents obtained from Morgan Chemicals Co. Cairo, Egypt. Thirty-six adult normal male albino rats Sprague Dawley strain weighing170±5g were obtained from Vaccine and Immunity Organization, Ministry of Health, Helwan Farm, Cairo, Egypt.

Methods

Preparation of psyllium seeds

The seeds of psyllium were ground to pass through 1.6 mm sieve then packed in polyethylene bags and stored in a freezer until used.

Basal diet composition.

The basal diet was formulated according to AIN-93M diet (Reeves et al., 1993) as follow: protein (10%), corn oil (10%), vitamin mixture (1%), mineral mixture (4%), choline chloride(0.2%), methionine (0.3%), cellulose (5%), and the remained is corn starch (69.5%). The vitamins and salts mixture were formulated according to Hegested et al., (1941) and Drury and Wallington, (1980) respectively.

Induction of diabetes

Diabetes were induced in Thirty- six normal healthy rats by injection into operationally with freshly prepared alloxan monohydrate in saline at a dose level of 150 mg/ kg body weight (Lazarow and Palay, 1954). Immediately after injection animals were received 5% glucose solution over night to overcome drug induced hypoglycemia (Wohaieb and Godin, 1987). After five days fast blood glucose (FBG) was analyzed using a specific kit by a drop of blood was obtained from tail vein and subjected to a strip of haemoglucose test. Rats with fasting blood glucose >150 mg/ dl were diabetic (Neeli et al., 2007).

Experimental Design

Rats were randomly divided into two main groups after acclimatization period (7 days), the first, negative control group (n = 6), fed on basal diet and the second group (diabetic rats, n = 30). Second group was divided into 5 subgroups (6 rats each). First subgroup is positive control fed on basal diet, the second and third subgroups fed on basal diet supplemented with 3 and 5% psyllium seeds respectively and fourth and fifth subgroups fed on basal diet supplemented with 3 and 5% psyllium husks respectively. Rats were housed in stainless steel wire cages. Water was introduced as well as ad-libitum diet for four weeks. At the end of the experimental, rats were fasted for 12-h then scarified. Blood samples were collected from the portal vein into dry clean centrifuge tubes for serum separation, blood samples centrifuged for 10 minutes at 3000 rpm to separate the serum. Livers of sacrificed rat were kept in 10% formalin solution till processed for histopathological examination.

Biochemical Analysis

Serum glucose, insulin and glycated hemoglobin A1C (HbA1c) were estimated according to Trinder, (1969), Temple et al., (1992) and Trivelli et al., (1971). Total cholesterol (TC), triglyceride (TG) and high-density lipoprotein (HDL-c) were determined by using methods of Thomas, (1992), Fassati and Prencipe, (1982) and Fredewaid, (1972) respectively. Low density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c) were calculated according to Lee and Nieman, (1996) as the following equation: LDL-c = Total cholesterol-(HDL-c + TG/5), VLDL-c = TG/5.

Serum alanine aminotransferase (ALT), aspartate amino trans ferase (AST) and alkaline phosphatase (ALP) were determined according to the methods described by Reitman and Frankel, (1975) Hafkenscheid (1979) and Moss (1982) respectively. Urea, uric acid and creatinine were determined according to the methods of Patton and Crouch, (1977), Barham and Trinder, (1972)., and Henry, (1974) respectively.

Histopathological Examination

Pancreas of the scarified rat washed in slain solution stored in formalin solution 10% for histopathological testing according to method mentioned by Bancroft et al., (1996).

Statistical Analysis

Results were expressed as the mean ± SD. Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a post hoc test according to the statistical package program (Armitage and Berry, 1987).

Results and Discussion

The effect of psyllium seeds and husks on serum glucose, insulin and HbA1C are shown in Table (1). The results showed that there was significant increase ($P \leq 0.5$) in serum glucose and significant decrease ($P \leq 0.5$) in serum insulin levels in positive control group compared to negative control group. Supplementation rat diets with 5 % of psyllium seeds, 3 and 5 % husks improved serum glucose levels in diabetic rats compared with positive control. Moreover, the best serum glucose was recorded for diabetic rats treated with psyllium husks by 5%. These results are agreement with Karhunen et al. (2010) who found that psyllium husks and its seeds fiber enriched meals improve glucose level significantly than non- fiber enriched meals (Pal et al., 2014). Also, Kalaiarasi and Pugalendi, (2009) and Mohammed et al., (2015) reported that the hypoglycemic activity of psyllium husks and its seeds may be due to the inhibition of liver gluconeogenesis. In the same table, there was no significant difference ($P \leq 0.5$) in insulin and HbA1C among positive control group and groups treated with 3 and 5 % of psyllium seeds and husks. Also, groups treated with 3 and 5 % of psyllium seeds and husks had lower values than positive control group. Abutair et al., (2016) reported that After 8 weeks of intervention, psyllium showed proven to improve HbA1c (8.5 to 7.5 %) when compared with the control group.

Serum total cholesterol and TG in normal and diabetic rats fed on diets with psyllium husks and seeds were recorded in Table (2). Positive control group had a significant increase in serum concentration of TG compared to negative control group and diabetic rats fed on diets with psyllium husks and seeds. Rats fed on diets with psyllium seeds and

husks at levels 3 and 5 % had decrease values in serum concentration of TG as compared to the positive control group.

Table (1): Effect of psyllium seeds and husks on serum glucose, insulin and HbA1c of diabetic rats

Groups		Glucose (mg/dl)	Insulin (mg/dl)	HbA1c (mg/dl)
Control (-)		169.3f ± 2.32	0.30 a ± 0.14	3.46b ± 0.32
Diabetic groups	Control (+)	260.3a ± 3.67	0.17 b ± 0.25	4.30a± 1.17
	psyllium seeds (3%)	244.3 b ± 0.75	0.20 b ± 0. 17	3.86ab±0.62
	psyllium seeds (5%)	206.3 c ± 1.75	0.18 b ± 0.15	3.76ab ±1.12
	Psyllium husks (3%)	188.0 d ± 1	0.17 b ± 0.08	4.03 a ±1.30
	Psyllium husks (5%)	174.3e ± 1.26	0.17 b ± 0.11	4.03a ± 0.28
LSD		3.89	0. 026	0.363

Mean values are expressed as means ± SD. Means with different superscript letters in the same column are significantly different at (P≤ 0.05). HbA1c: glycated hemoglobin A1C.

Also, supplementation rat diets with 3 and 5% of psyllium seeds and husks had the same effect on TC and TG levels. Supplementation rat diets with 5% of psyllium husks were more effective in decreasing TC than rats fed with 3 and 5% of psyllium seeds and 3% of psyllium husks. Liu, et al., (2004) and Zakia et al., 2018) concluded that psyllium is a very useful dietary fiber in terms of lowering cholesterol.

Table (2): Effect of psyllium seeds and husks on TC and TG of diabetic rats

Groups		TC (mg/dl)	TG (mg/dl)
Control (-)		60.0 b ± 2.11	30.3 c ± 3.15
Diabetic groups	Control (+)	69.3 a ± 1.52	60.3 a ± 9.074
	psyllium seeds (3%)	64,0 ab ± 1.2	443 b ± 3.51
	psyllium seeds (5%)	66,0 ab ± 3.60	42.7 b ± 3.511
	Psyllium husks (3%)	66,0 ab ± 4.58	48.3 b ± 5.85
	Psyllium husks (5%)	62,0 b ± 3.4	41.7 b ± 1.15
LSD		5.768	7.913

Mean values are expressed as means ± SD. Means with different superscript letters in the same column are significantly different at P≤ 0.05. TC: total cholesterol, TG: triglycerides

Data in table (3) indicate that positive control group had a significant increase in serum VLDL-c and LDL-c compared with negative control while, HDL-c had opposite trend. Supplementation rat diets with 3 and 5% of psyllium seeds and 3% of psyllium husks did not differ in their effect on HDL, LDL and VLDL-c. Also, the best serum HDL and LDL were recorded for diabetic rats treated with psyllium husks at 5%. Singh et al., (2007) reported that feeding diabetic rats on diets supplemented with psyllium husks and seeds showed a significant decrease in serum levels of LDLc and VLDLc and increases in the level of HDLc. Also, Shrestha (2007) indicated that psyllium extract significantly reduced serum LDL and VLDL levels ($p \leq 0.01$). The hypolipidemic effect of psyllium seeds and husks doesn't seem to be due to only one component, but rather to the synergetic action of its different constituents, including soluble fiber, phenolic compound, flavonoids, oleic, linoleic, linolenic, caffeic acids and chlorogenic acid. Flavonoids may act by making liver cells more efficient to remove LDL-C from blood by increasing LDL-C receptor densities in liver and by binding to apo-lipoprotein B (Gunness et al., 2010).

Table (3): Effect of psyllium seeds and husks on HDL-c, LDL-c and VLDL-c of diabetic rats

Groups		HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control (-)		28.3 a ± 2.5	25.6 b ± 2.2	6.1 c ± 0.7
Diabetic groups	Control (+)	18.0 b ± 4.0	39.4 a ± 2.6	11.9 a ± 1.7
	psyllium seeds (3%)	20.0b ± 2.0	35.1 a ± 2.4	8.9 b ± 0.7
	psyllium seeds (5%)	20.7b ± 3.1	36.81 a ± 5.9	8.5 b ± 0.7c
	Psyllium husks (3%)	22.7b ± 0.6	34.01 a ± 3.6	9.3 b ± 0.6
	Psyllium husks (5%)	27.7 a ±2.5	26.01b ± 5.1	8.3 b ± 0.2
LSD		4.90	7.72	1.38

Mean values are expressed as means ± SD. Means with different superscript letters in the same column are significantly different at P≤ 0.05. HDL: high density lipoprotein, LDL: low density lipoprotein, VLDL: very low-density lipoprotein

Data in table (4) indicated that control positive group was significantly higher in serum levels of urea and uric acid but non-significantly increase the level of serum creatinine when compared with control negative group. Rats treated with psyllium seeds showed non-significantly While psyllium husks showed significantly lower values in serum levels of urea. As for creatinine and Uric Acid showed non-significantly decrease values compared to the positive control group. On the other hand rats fed psyllium seeds and husks on groups 3,4,5 and 6 showed nonsignificant differences between them as for uric acid and creatinine Also, the best serum urea and Uric Acid were recorded for diabetic rats treated with psyllium husks. In study Martin et al., (2012) showed that treated fatty liver rats with Psyllium recorded a high significant decrease ($P \leq 0.01$) in serum urea and

creatinine when compared with control group. Also, Elhardallou et al., (2015) reported that leaves of C.intybus significantly reduced the level of serum urea in waster-albino rats.

Table (4): Effect of psyllium seeds and husks on serum kidney functions of diabetic rats

Groups		Urea (mg/dl)	Uric Acid (mg/dl)	Creatinine (mg/dl)
Control (-)		25.3 b± 2.8	1.3 b ± 0. 1	0.6 a ± 0
Diabetic groups	Control (+)	34.6 a ± 4.5	1.7 a ± 0.1	0.7 a ±0.1
	psyllium seeds (3%)	33.3 a ± 3.4	1.4 ab ± 0.1	0.6 a ±0.1
	psyllium seeds (5%)	28.2 ab ±1.8	1.4 ab ± 0.1	0.7 a ±0.1
	Psyllium husks (3%)	27.7 b ± 1.8	1.6 a ± 0. 1	0.7 a ± 0.1
	Psyllium husks (5%)	27.6 b ± 1.1	1.5 ab ± 0.2	0.7 a ±0.1
LSD		5.42	0.232	0.187

Values are expressed as mean ± SD. Means in the same column with different superscript letters are significantly different ($p \leq 0.05$).

Data in table (5) indicate that positive control group had a significant increase in serum ALT, AST and ALP compared with negative control. Supplementation rat diets with 5 % of psyllium seeds, 3 and 5 % husks improved serum ALT, AST and ALP in diabetic rats compared with positive control. Supplementation rat diets with 3 and 5% of psyllium seeds and 3% of psyllium husks did not differ in their effect on ALT, AST and ALP. Also, the best serum ALT were recorded for diabetic rats treated with psyllium seeds at 5%.While, the best serum AST and ALP were recorded for diabetic rats treated with psyllium husks at 5%. These results are in agreement with Elhardallou et al., (2015) founded that feeding on diet supplemented with psyllium, alone and combined, for four weeks to diabetic rats significantly decreased the levels of ALT, AST, and ALP enzymes in the serum compared with control positive group. Also, Zakia et al., (2018) they reported that Treatment with Psyllium extract significantly reduced ALT, AST and ALP enzymes after 4 weeks of treatment implying that the plant has executed a protective effect of liver damage. This improvement may be due to the antioxidant agent's activity of psyllium.

Histopathological examination of liver

Fig (1) showed the effect of psyllium seeds and husks on histological examination of liver tissue of diabetic rats. Microscopical examination of liver sections of negative control

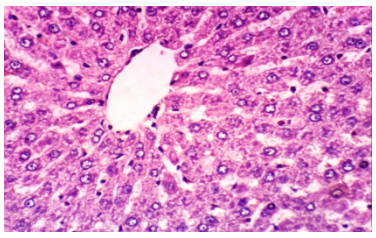
rats revealed normal histological structure of hepatic lobule while, positive control showed focal hepatocellular necrosis associated with inflammatory cells infiltration and hepatocellular vacuolar degeneration and portal infiltration with mononuclear cells. On the other hand, the results showed that liver of rats from group 3 (treated with psyllium seeds by 3%) revealed small focal hepatocellular necrosis associated with inflammatory cells infiltration.

Table (5). Effect of psyllium seeds and husks on serum liver functions enzymes of diabetic rats.

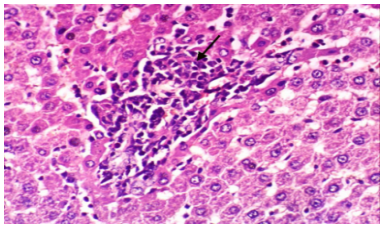
Groups		ALT (u/l)	AST (mg/dl)	ALP (mg/dl)
Control (-)		47.0c ±1.2	126.7 e± 2.5	149.0c± 19.0
Diabetic groups	Control (+)	79.0a± 1.7	203.0a± 9.2	316.6a± 39.0
	psyllium seeds (3%)	61.0b± 7.2	183.6b± 2.5	225b± 2.0
	psyllium seeds (5%)	48.7 c± 3.2	172.6 c± 2.5	215.3 b± 9.5
	Psyllium husks (3%)	50. 7c ±7.5	174.3bc± 5.5	169.6c± 9.7
	Psyllium husks (5%)	52.7bc ± 3.1	151.0d ±5.6	147.6c± 21.0
LSD		8.93	10.28	40.68

Values are expressed as mean ± SD. Means in the same column with different superscript letters are significantly different ($p \leq 0.05$). ALT: Alanine Aminotransferase. AST: Aspartate Aminotransferase. ALP: Alkaline Phosphatase

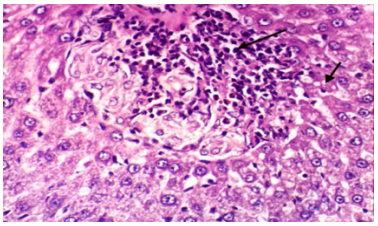
Fig (1): Effect of psyllium seeds and husks on histological examination of liver tissue of diabetic rats



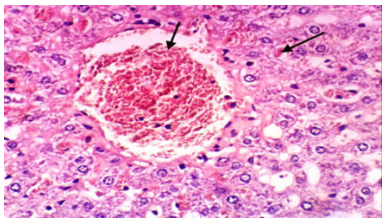
Negative control



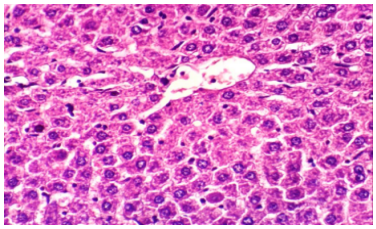
Positive control



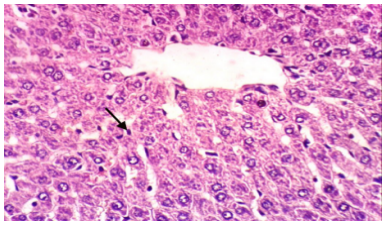
Psyllium Seeds 3%



Psyllium Seeds 5%



Psyllium Husk 3%



Psyllium Husk 5%

Meanwhile, sections from group 4(treated with psyllium seeds by 5%) showed congestion of central vein and hepatic sinusoids and portal infiltration with mononuclear cells. Also, liver of rats from group 5 (treated with psyllium husks by 3%) showed mild changes as slight Kupffer cells activation, congestion of central veins and hepatic sinusoids with slight vacuolization of some hepatocytes. However, liver of rats from group 6 (treated with psyllium husks by 5%) revealed apparent normal hepatic lobule with congestion of central vein in some sections. This result was agreed with those of Ponrasu et al. (2018) and Zakia et al. (2018) reported that the Histopathological observations in liver rat's tissues revealed that psyllium was non-toxic.

Conclusion

The results of the current study showed that the diet supplemented with psyllium seeds and husks showed hypoglycemic effect while insulin and Hb Ac1 did not improve. Therefore, it was concluded that using psyllium seeds and husks in high doses than current and for a long period may be useful.

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التأثيرات المحتملة لبذور السيليوم وقشورها على الفئران المصابة بمرض السكر

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الملخص العربى :

أجريت هذه الدراسة لمعرفة التأثيرات المحتملة لبذور السيليوم وقشورها على الفئران المصابة بمرض السكري. تم تقسيم ستة وثلاثين من ذكور الفئران البيضاء (170 ± جم) إلى مجموعتين رئيسيتين. المجموعة الضابطة الأولى سالبة (ن = 6) ، تتغذى على الوجبة الغذائية الأساسية والمجموعة الثانية (الفئران المصابة بالسكري ، ن = 30). تم تقسيم المجموعة الثانية إلى 5 مجموعات فرعية (6 فئران لكل مجموعة). المجموعة الفرعية الأولى عبارة عن مجموعة ضابطة موجبة تم تغذيتها على الوجبة الغذائية الأساسية ، وتتغذى المجموعتان الفرعيتان الثانية والثالثة على الوجبة الغذائية الأساسية المضاف إليه 3 و 5٪ بذور سيلليوم على التوالي والمجموعة الفرعية الرابعة والخامسة تغذيان على الوجبة الغذائية الأساسية المضاف إليه 3 و 5٪ قشور سيلليوم على التوالي. في نهاية الفترة التجريبية (30 يومًا) ، تم تخدير الفئران للحصول على عينات الدم. تم تقدير مستوى جلوكوز الدم ، الأنسولين ، الهيموجلوبين السكري (HbA1C) ، مستوى الدهون ، وظائف الكبد والكلى. تم إجراء فحص هستوباثولوجي للكبد. تغذية الفئران المصابة بمرض السكر بالوجبة الاساسية المدعمة ببذور أو قشور السيليوم أدى إلى انخفاض كبير في مستويات جلوكوز الدم والدهون الثلاثية والكوليسترول منخفض الكثافة جدا ووظائف الكبد. في حين لم يكن هناك فرق معنوي ($P \leq 0.5$) في الأنسولين و HbA1C والكوليسترول منخفض الكثافة والكوليسترول مرتفع الكثافة ووظائف الكلى بين المجموعة الضابطة الموجبة والمجموعات المعالجة بـ 3 و 5٪ من بذور وقشور السيليوم. تغذية الفئران بالوجبة المعاملة بقشور السيليوم (5٪) كانت أكثر فاعلية من تلك المعاملة بقشور السيليوم (3٪) وبذور السيليوم (3 و 5٪) في تحسين انسجة للكبد. زيادة الجرعات يمكن أن تصبح أكثر وضوحا.

الكلمات المفتاحية: السيليوم، الأنسولين، وظائف الكبد، الهيموجلوبين السكري.