

Investigation of Anti-diabetic and Hypocholesterolemic Potential of *Psyllium* Husk Fiber (*Plantago psyllium*) in Diabetic and Hypercholesterolemic Albino Rats

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Abstract—The present study was conducted to observe the effect of *Plantago psyllium* on blood glucose and cholesterol levels in normal and alloxan induced diabetic rats. To investigate the effect of *Plantago psyllium* 40 rats were included in this study divided into four groups of ten rats in each group. One group A was normal, second group B was diabetic, third group C was non diabetic and hypercholesterolemic and fourth group D was diabetic and hypercholesterolemic. Two groups B and D were made diabetic by intraperitoneal injection of alloxan dissolved in 1mL distilled water at a dose of 125mg/Kg of body weight. Two groups C and D were made hypercholesterolemic by oral administration of powder cholesterol (1g/Kg of body weight). The blood samples from all the rats were collected from coccygial vein on 1st day, then on 21st and 42nd day respectively. All the samples were analyzed for blood glucose and cholesterol level by using enzymatic kits. The blood glucose and cholesterol levels of treated groups of rats showed significant reduction after 7 weeks of treatment with *Plantago psyllium*. By statistical analysis of results it was found that *Plantago psyllium* has anti-diabetic and hypocholesterolemic activity in diabetic and hypercholesterolemic albino rats.

Keywords—Albino rats, alloxan, *Plantago psyllium*, statistical analysis

I. INTRODUCTION

THE history of diabetes mellitus dates back to ancient Egypt, where its symptoms were described around 2000 B.C. The word diabetes means Siphon which describes a major symptom of the condition i.e. frequent urination and mellitus means honey, and depicts one of the early signs of diabetes i.e. sugar in the urine [1]. From all over the world several millions of population gets affected from Diabetes mellitus, a complex syndrome involving severe insulin dysfunction in conjugation with gross abnormalities in glucose homeostasis and lipid metabolism. The individuals with diabetes have a 25-fold increased risk of blindness, a 20-fold increased risk of renal failure, a 20-fold increased risk of amputation as a result of gangrene and a 2 to 6 fold increased risk of coronary heart disease and ischaemic brain damage [2]. Diabetes may also be associated with genetic syndromes, surgery, drugs, malnutrition, infections and other illnesses [3]. Cholesterol is a soft, waxy substance found among the lipids (fats) in the bloodstream and in all animal body's cells.

It is an important part of a healthy body because it is used to form cell membranes, some hormones and is needed for other functions. But a high level of cholesterol in the blood i.e. hypercholesterolemia is a major risk factor for coronary heart disease, that leads to heart attack [4]. Many plant extracts and plant products have shown association with the treatment of several ill-fated diseases including diabetes [5]. Compounds from their natural formulations are more active rather than their isolated form [6]. Presently control of diabetes mellitus also relies on many chemicals and plant extracts [7]. More than 400 traditional plants treatments for diabetes mellitus Type-II have been recorded, but only a small number of these have received scientific and medical evaluation to assess their therapeutic efficacy. There was a gap in proper understanding of medicinal plants for mankind in the past because traditional medicines generally lacked scientific explanations [8]. *Plantago psyllium* is found in India, Northern Africa and Pakistan. It has been used traditionally for constipation, diarrhea, haemorrhoids, irritable bowel syndrome, weight loss, obesity, high cholesterol and diabetes [9]. The seeds and husks contain high levels of fiber and become highly gelatinous when soaked in water. This stimulates bowel evacuation and thus has been used widely as a fiber supplement in the treatment of constipation [10]. The plant has been shown to reduce carbohydrate absorption [11]. It decrease delay in gastric emptying and reduces colon transit time in man. *Psyllium*, also referred to as ispaghula (or isphagula), is derived from the husks of the seeds of *Plantago ovata*. *Psyllium* contains a high level of soluble dietary fiber, and is the chief ingredient in many commonly used bulk laxatives, including products such as *Metamucil* and *Serutan*. *Psyllium* has been studied as a "non-systemic" cholesterol-lowering agent, with generally modest effects seen on total cholesterol and low-density lipoprotein levels [12]. *Psyllium ovata* reduces total cholesterol and LDL-cholesterol in animals [13, 14] and in man [15, 16]. Consumption of diets high in fiber reduces the risk factors of cardiovascular disease (CVD) by acting, in part, on plasma lipid concentrations [13, 17]. Numerous studies have shown that soluble fibers are more effective in lowering blood cholesterol than insoluble fibers [13, 18]. Current suggestion is to increase the amount of dietary fiber, specifically of soluble (viscous) fiber, to 10–25 g/d to more effectively lower cholesterol concentrations [19]. Thus, an increased soluble fiber intake, within a therapeutic

lifestyle, takes on an essential modality in the clinical management of CVD risk reduction [20].

II. MATERIALS AND METHODS

Plantago Psyllium

Plantago psyllium was collected from the local market of Lahore. The leaves of *Plantago psyllium* were sun and oven dried (60°C) and ground to make powder and stored in air tight polyethylene bags to keep it moisture free till the time of use.

Cholesterol

1% (1g/Kg diet) cholesterol powder was fed to the albino rats orally for 10 days as prescribed by Reeves *et al* [21].

Alloxan

Alloxan induced hyperglycemia has been described as a useful experimental model to evaluate the activity of hypoglycemic agents [22]. Diabetes was induced by a single intraperitoneal injection of alloxan prepared in 0.1mol/L citrate buffers at a dose of 100 mg/Kg body weight. Diabetes was confirmed in the alloxan treated rats by measuring the fasting blood glucose concentration 8-10 days post-injection.

Enzymatic Kits

Commercial kits of the company Randox were used to determine serum glucose and serum lipid levels in albino rats by spectrophotometer.

Animals

Total 40 albino rats (*Rattus norvegicus*) of either sex weighing between 200-300 g were used in this study. These animals were housed in steel cages under controlled laboratory conditions. They were maintained on standard pellet diet and were given water ad-labium.

Experimental Design and Induction of Diabetes

The animals were divided into four groups (table I) and each group consisted of ten rats. Diabetes was induced by a single intra peritoneal injection of alloxan prepared in 0.1mol/L citrate buffer at a dose of 100 mg/Kg body weight. The control rats were only injected with freshly dissolved 0.1mol/L citrate buffer (pH 4.5). The rats with blood glucose level above 150mg/dl were considered as diabetic and were used in the further experiments. Diabetes was confirmed in the alloxan treated rats by measuring the fasting blood glucose concentration 8–10 days post-injection as reported by [23]. The rats were made hypercholesterolemic by oral administration of 1% cholesterol powder (1g/Kg body weight) for 10 days as prescribed by Reeves *et al* [21]. Then, the hypercholesterolemic condition was confirmed by using respective diagnostic kits at the 11th day of experiment. After

the confirmation of diabetic and hypercholesterolemic conditions of rats, the day on which rats were treated with *plantago psyllium* powder at a dose of 0.5mg/Kg body weight was considered as 1st day of experiment.

TABLE I
ANIMAL GROUPING

| Groups | Treatment |
|--|---|
| Group A: Normal (Control) | Normal Feed |
| Group B: Diabetic | Normal Feed and <i>plantago psyllium</i> (0.5g/Kg of body weight) |
| Group C: Non Diabetic and Hypercholesterolemic | Normal Feed and <i>plantago psyllium</i> (0.5g/Kg of body weight) |
| Group D: Diabetic and Hypercholesterolemic | Normal Feed and <i>plantago psyllium</i> (0.5g/Kg of body weight) |

Blood Collection and analysis

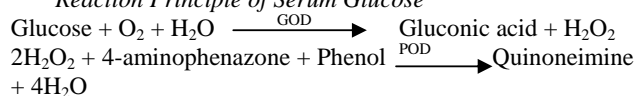
Blood was taken from the coccygial vein of overnight fasted albino rats. Collected blood poured into centrifuged glass tubes, and then centrifuged at 3500g for ten minutes and serum was separated and stored in a deep freezer for further biochemical measurements. The specific enzymatic kits were used to assess serum lipid profile and glucose levels of rats using spectrophotometer.

Estimation of Serum Glucose and Serum Cholesterol

Principle of Serum Glucose

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as an indicator.

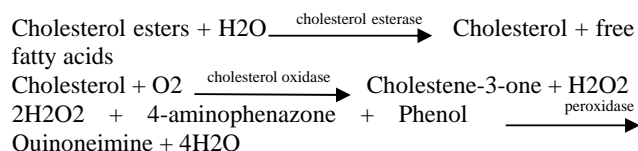
Reaction Principle of Serum Glucose



Principle of Serum Cholesterol

Cholesterol is determined after enzymatic esterification and oxidation in the presence of cholesterol esterase and cholesterol oxidase. The hydrogen peroxide formed reacts under catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye as indicator.

Reaction principle of Serum Cholesterol



Procedure

Two tubes out of 9 test tubes were labeled as blank and standard. Remaining 7 tubes were labeled as 1, 2, 3... 7 for each sample of rat's serum from each group. 1mL reagent was taken in all the tubes by pipette. 10 µl of standard solution from the kit was added to the tube labeled as standard and 10 µl of each serum sample was taken in tubes labeled as 1, 2, 3,....7. Contents of all the tubes were mixed well and then incubated at 37°C for 10 minutes. After incubation, the absorbance of standard (AbsStd) and sample (AbsS) was measured at the wavelength of 546 nm against the blank (AbsSRB).

Calculations

The concentration of glucose and cholesterol in serum was calculated by the following formula as prescribed by Mackey and Macky [24].

Glucose concentration (mg/dl) = (A test / A standard) × 100

Statistical analysis

Descriptive statistics mean and standard deviation were calculated for all the variables of each group. ANOVA was applied for statistical analysis using statistical software Minitab V. 1.5 and p value at <0.05 had been considered significance level Steel *et al.* [25].

III. RESULTS

Present research work was conducted in order to evaluate the anti-diabetic and hypocholesterolemic activity in diabetic and hypercholesterolemic albino rats

Serum Glucose Levels (mg/dl)

The change in serum glucose levels of rats due to *Plantago psyllium* treatment of 7 weeks is presented in table II. The average blood glucose levels of group A were recorded as 103 mg/dl on 1st day of experiment. The glucose level changed to 104 mg/dl and 105 mg/dl at day 21st and the day 42nd of the experiment respectively while they were not treated with *Plantago psyllium*. The overall reduction in the glucose levels was observed which was inconsistent and non-significant (P>0.05) table II.

The average blood glucose levels of rats of group B were recorded as 215 mg/dl on 1st day of the experiment before the treatment with *Plantago psyllium* powder. The glucose levels after the treatment with *Plantago psyllium* powder decreased to 202 mg/dl and 178 mg/dl at day 21st and the day 42nd of the experiment respectively. The overall decrease was observed in blood glucose levels which was consistent and significant (P<0.05) table II.

The average blood glucose levels observed in of rats of group C were recorded as 105 mg/dl on 1st day of experiment. They were treated with *Plantago psyllium* powder. They changed to 100 mg/dl and 95 mg/dl at day 21st and the day 42nd of the experiment respectively. The overall decrease in the blood glucose levels was observed for this group which was consistent and significant (P<0.05) table II.

The average blood glucose levels of rat of group D were recorded as 196 mg/dl on 1st day of experiment. The blood glucose levels for this group were changed to 184 mg/dl and 172 mg/dl at day 21 and the day 42 of the experiment respectively after the treatment with *Plantago psyllium* powder. The overall decrease in blood glucose levels was found which was significant (P<0.05) table II.

TABLE II
SERUM GLUCOSE LEVELS (MG/DL) OF RATS DUE TO *Plantago psyllium* TREATMENT

| Groups and Treatments | Glucose Level (mg/dl) Means ± S.D | | | Significance |
|--|--------------------------------------|---------------|----------------|--------------|
| | Day1 | Day21 | Day 42 | |
| Group A: Non Diabetic untreated | 103± 5.16 | 104± 6.20 | 105 ± 4.46 | 0.150* |
| Group B: Diabetic treated with <i>P. psyllium</i> | 215± 9.15 | 202± 7.28 | 178 ± 8.58 | 0.001° |
| Group C: Non Diabetic and hypercholesterolemic treated with <i>P. psyllium</i> | 105± 5.12 | 100± 5.12 | 95 ± 5.21 | 0.003° |
| Group D: Diabetic and hypercholesterolemic treated with <i>P. psyllium</i> | 196± 10.50 | 184± 10.50 | 172 ± 10.50 | 0.011° |

* Non Significant

° Significant

Total Serum Cholesterol

The change in serum cholesterol levels of rats due to *Plantago psyllium* treatment of 7 weeks is presented in table III. The average serum cholesterol levels of group A were recorded as 132mg/dl on 1st day of experiment. The serum cholesterol level changed to 131 mg/dl and 133 mg/dl at day 21st and the day 42nd of the experiment respectively while they were not treated with *Plantago psyllium*. The overall reduction in the serum cholesterol levels was observed which was inconsistent and non-significant (P>0.05) table III. The average serum cholesterol levels of rats of group B were recorded as 213 mg/dl on 1st day of the experiment before the treatment with *Plantago psyllium* powder. The serum cholesterol levels after the treatment with *Plantago psyllium* powder decreased to 203 mg/dl and 193 mg/dl at day 21st and the day 42nd of the experiment respectively. The overall decrease was observed in serum cholesterol levels which was consistent and significant (P<0.05) table III. The average

serum cholesterol levels observed in of rats of group C were recorded as 269 mg/dl on 1st day of experiment. They were treated with *Plantago psyllium* powder. They changed to 259mg/dl and 249mg/dl at day 21st and the day 42nd of the experiment respectively. The overall decrease in the serum cholesterol levels was observed for this group which was consistent and significant ($P < 0.05$) table III. The average serum cholesterol levels of rat of group D were recorded as 313mg/dl on 1st day of experiment. The serum cholesterol levels for this group were changed to 298mg/dl and 281 mg/dl at day 21st and the day 42nd of the experiment respectively after the treatment with *Plantago psyllium* powder. The overall decrease in serum cholesterol levels was found which was significant ($P < 0.05$) table III.

TABLE III
SERUM CHOLESTEROL LEVELS (MG/DL) OF RATS DUE TO *Plantago psyllium* TREATMENT

| Groups and Treatments | Cholesterol Level (mg/dl) Means \pm S.D | | | Significance |
|--|--|-----------------|-----------------|--------------|
| | Day 1 | Day 21 | Day 42 | |
| Group A: Non Diabetic untreated | 132 \pm 9.53 | 131 \pm 9.63 | 133 \pm 9.80 | 0.168* |
| Group B: Diabetic treated with <i>P. psyllium</i> | 213 \pm 12.84 | 203 \pm 12.6 | 193 \pm 12.68 | 0.001° |
| Group C: Non Diabetic and hypercholesterolemic treated with <i>P. psyllium</i> | 269 \pm 9.94 | 259 \pm 9.94 | 249 \pm 9.94 | 0.012° |
| Group D: Diabetic and hypercholesterolemic treated with <i>P. psyllium</i> | 313 \pm 12.75 | 298 \pm 12.94 | 281 \pm 12.51 | 0.033° |

* Non Significant

° Significant

IV. DISCUSSION

Considering the importance of natural products, the present study was designed to investigate the antidiabetic and hypocholesterolemic effects of feeding *Plantago psyllium* (Isphaghol husk) on serum glucose and cholesterol level in Diabetic Albino rats. Recent clinical trials have highlighted the importance of aggressive plasma lipid lowering for both the primary and secondary prevention of atherosclerotic events in patients with diabetes [26].

Control of diabetes by spices and other natural products is becoming popular and more economical for use in developing countries. Spices come from the dried aromatic plants or trees and may be the bark, fruit, bud or the berry of trees/plants [27]. Many herbal medicines have been recommended for the treatment of diabetes [28].

Glucose level of diabetic rats of Group B and Group D decreased from 213.5 mg/dl to 188.5 mg/dl and 196.1 mg/dl to 172.1 mg/dl respectively having $P < 0.001$ as shown in table

2. These results are in conformity with the work of Moharib and Bartan [29] they found a significant decrease in the levels of glucose by administering dietary fibers to the diabetic rats. Similarly reduction in glucose and cholesterol levels was observed in diabetic patients without significant adverse effects by Rodriguez *et al* [30]. No significant changes were observed in the patient's weight for both groups (not significant). Fasting plasma glucose, total cholesterol, LDL cholesterol, triglycerides levels, showed a significant reduction ($p < 0.05$), whereas HDL cholesterol increased significantly ($p < 0.01$) following *Plantago psyllium* treatment.

Plantago psyllium seed have effect on plasma cholesterol of hypercholesterolemic rats and diabetic rats. The results given in table 3 showed that the plasma cholesterol level 213.2 mg/dl to 193.7mg/dl in Group B and 313.4 mg/dl to 281.5mg/dl in Group D. The reduction in plasma cholesterol level was significantly relevant to the findings of Kritchevsky *et al* [31]. They found that *P. psyllium* husk virtually normalized liver size and produce lower serum total cholesterol level and higher HDL-Cholesterol than observed in normal controls. Similarly it was reported by Ziai *et al* [32] that *P. psyllium* husk fiber has lowering effects on lipids and glucose level in patients with type 2 diabetes. They showed significant reduction ($p < 0.05$) in fasting glucose plasma level and high density cholesterol level decreased significantly ($P < 0.05$).

Our results are also inconsistent Arjmandi *et al* [33] where they found that whether the storage conditions and the levels of *P. psyllium* in the diet modulate its hypercholesterolemic effects. The cholesterol lowering activity of *P. psyllium* is unaltered by storage conditions shown to cause a moderate degree of hydrolysis. Non diabetic and hypercholesterolemic rats treated with *P. psyllium* showed a decrease in the fasting plasma glucose level and cholesterol levels the decrease was non-significant with respect to glucose level ($P < 0.061$) while the cholesterol level significantly lowered ($P < 0.001$) in the studies conducted by Arjmandi *et al* [33] indicated that the cholesterol lowering activity of *Plantago psyllium* in male Sprague-Pawley rats was very effective and unaltered by storage conditions. Similarly Abraham and Mehta [11] conducted a study to determine the effect of *Plantago psyllium* husk on plasma total and lipoprotein cholesterol in healthy human subjects and to elucidate possible hypocholesterolemic mechanisms.

V. CONCLUSION

The present research work reveals that the the oral administration of *Plantago psyllium* powder showed hypoglycemic and hypocholesterolemic effects in alloxan induced diabetic rats, therefore it was concluded that *Plantago psyllium* powder is helpful to lower glucose and cholesterol level in treatment of hyperglycemia and hypercholesterolemia.

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