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RESEARCH ARTICLE

Lipid Lowering Efficacy of *Pennisetum glaucum* Bran in Hyperlipidemic Albino Rats

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ABSTRACT

The objective of the study was to determine lipid lowering efficacy of *Pennisetum (P) glaucum* (Pearl millet, locally known as bajra), bran in hyperlipidemia albino rats. Simvastatin, (Tablet survive®), was used as cholesterol lowering synthetic drug. The period of 0-15 days was considered as a lead-in period to induce hyperlipidemia with atherogenic diet in albino rats. *P. glaucum* bran at dose rate of 2, 4 and 6 g/kg BW showed lipid lowering efficacy in hyperlipidemic rats at post-treatment days 30, 45 and 60. At the level of 6 g/kg, *P. glaucum* bran was able to produce a significant ($P<0.05$) increase in HDL- cholesterol (47%) and fall in other lipid profile parameters i.e. total lipids (41%), triglycerides(48%), total cholesterol (39%) and LDL- cholesterol (55%). *P. glaucum* 6 g/kg also reduced total cholesterol in liver tissue and increased fecal bile acid secretion. The results of present study suggest that 6 g/kg *P. glaucum* bran and 0.6 mg/kg Simvastatin were equally effective in treating hyperlipidemia in albino rats. Moreover, the potency of *P. glaucum* for stimulating fecal bile acid secretion in albino rats may safely be conceived, at least, as a part of mechanisms for its antihyperlipidemic efficacy.

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INTRODUCTION

Increasing interests in atherosclerosis prevention and identification of hyperlipidemia as risk factor for ischemic heart disease have stimulated the study that started a new era in the treatment of atherosclerosis. In the last two decades both retrospective and prospective studies have shown strong correlation between levels of circulating lipids and mortality rates from coronary atherosclerotic heart disease (Anwar *et al*., 1999). Synthetic drugs used as antihyperlipidemic agent shows serious side effects (Javed *et al*., 1994; Javed *et al*., 2002). Therefore, attempts were focused on the Ayurvedic system of medicine to find out the drugs having hypolipidemic activity without side effects (Miura *et al*., 2003; Wei *et al*., 2003).

Dietary fibers have been reported to increase excretion of fecal bile acids resulting a fall in serum cholesterol (Timothy *et al*., 2003). In this respect, cellulose, metamucil and pectin have been found to enhance fecal elimination of cholesterol as bile acids leading to fall in serum cholesterol (Daggy *et al*., 1994). Moreover, dietary fibers may also increase the bulk of contents of intestine and so sequestering and diluting bile acids. Further, shorter transit of contents of intestine may cause decreased absorption of bile acids in distal portion of ileum (Mukherjee, 2010).

Pennisetum glaucum, commonly known as pearl millet (Bajra), has been found to be appetizer and tonic. Its fruits have been reported as a useful therapy for open facial pimples (Chopra *et al*., 1986). *P. glaucum*, bran, has been considered a good source of dietary fiber (Nandini and Salimath, 2001). In view of these facts, the present project was designed for the pharmacological evaluation of *P. glaucum* bran for its antihyperlipidemic efficacy in albino rats.

MATERIALS AND METHODS

One hundred and eighty healthy adult male albino rats were purchased from the National Institute of Health, Islamabad, Pakistan. The animals were kept under the similar management conditions in animal room of Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, Pakistan. The rats were housed in iron cages at ambient temperature with a 12/12 h period of light/dark. After seven days of acclimatization, the rats were randomly divided into six equal groups. The average body weight of all groups of albino rats ranged from 200 to 300 g. The experiments were conducted with the prior approval by the Directorates of Research and Advanced Studies. The rats were sacrificed according to the rules

laid down by the Society of Ethics of Animals, University of Agriculture, Faisalabad, Pakistan.

The rats were provided with normal routine rat feed (rat chow) for the first week of their arrival. The feed was made available twice a day, usually in morning and evening. However, drinking water was available throughout 24 hours. Expect one group, kept on normal routine rat feed, the rest of the groups were provided with atherogenic diet for 0-15 days. The period of 0-15 days was considered as a lead-in period to induce hyper-lipidemia in albino rats. The atherogenic diet comprised of Cholesterol 0.5%, Coconut oil 20% and Cellulose 15%, mixed in normal routine rat feed (Aslam, 2005). Simvastatin, 20 mg (Tablet survive®), Werrick Pharmace- uticals, Islamabad, Pakistan) were used as cholesterol lowering synthetic drug.

The seeds of *P. glaucum* were procured from the Department of Botany, Faculty of Science, University of Agriculture, Faisalabad, Pakistan. The seeds were finely powdered with an electric grinder to get the flour of this medicinal plant. This flour was ultimately sieved to get the bran of *P. glaucum*. The bran was used for feeding to albino rats as an antihyperlipidemic indigenous medicinal plant drug. The drugs were thoroughly mixed with atherogenic diet and then fed to albino rats.

Before the onset of present investigations, pharmacological screening of *P. glaucum* bran for its antihyperlipidemic activity and dosage was carried out in albino rats. Feeding schedule of normal routine rat feed, atherogenic feed, *P. glaucum* bran and Simvastatin in albino rats during the experimental period of 0 to 60 days has also been presented in Table 1.

Blood samples were collected on 0, 15, 30, 45 and 60 post treatment days. On each sampling day, 6 albino rats were slaughtered from each group and blood of individual albino rat was collected. The samples were allowed to clot for 20 minutes at refrigeration temperature and then centrifuged at 1968 x g for 5 minutes. Serum thus separated was stored at freezing temperature till analysis.

At the completion of experiment, i.e. at post treatment day 60, the feces of rats of groups I, II, III and VI were collected separately. Afterwards, the rats of these groups were scarified and the liver of individual animals was removed free from fat and connective tissue. The fecal and liver samples were stored at -20°C until analysis.

Cholic acid excreted in the feces was analyzed following method of Reinhold and Wilson (1934). Total cholesterol was extracted from the liver by a method described by Choi (1991). Lipid profile parameters including total lipids, triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-Cholesterol) and low density lipoprotein cholesterol (LDL-Cholesterol) were determined in the serum and total cholesterol in the extracts of liver of rats with reagent kits (Randox Laboratories Ltd, UK).

The significance of the differences between the lead in period day 15 pre-treated and 30, 45 and 45 days post-treated values was tested using Student's T-test. To assess the antihyperlipidemic effect of *P. glaucum* bran on the cholesterol and cholic acid contents in the tissues of liver and feces, respectively, comparison between the treatment groups, treated control group and/or untreated control group was performed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMR). In all tests the criterion of statistical significance was ($P<0.05$).

RESULTS AND DISCUSSION

Hyperlipidemia was produced in normal healthy rats by feeding atherogenic diet from 0-15 days. Almost 2 times increase was produced in lipid profile parameters i.e. total lipids, triglycerides, total cholesterol and LDL-Cholesterol at day 15 than their respective values at day 0 while HDL-Cholesterol has been found to show decreasing trend 1-1.5 times as compared to day 0 (Fig. 1). However, 2.5-7 folds increase in lipid profile indicators have been reported in rabbit fed with atherogenic diet and cholesterol (400 mg/kg) for 90 days (Javed *et al.*, 2009). Furthermore, 3.5-9 times increase in these parameters has been reported in albino rabbits fed with atherogenic diet and cholesterol (400 mg/kg) for 120 days (Purohit and Daradka, 2001).

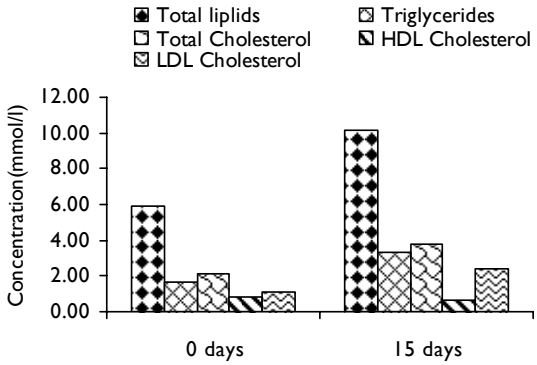

Fig. 1: Mean (mg/dl) values of serum lipid profile in albino rats fed with atherogenic diet.

After oral administration of *P. glaucum* bran at the doses of 2, 4 and 6 g/kg and Simvastatin at 0.6 mg/kg to hyperlipidemic albino rats, reductions in lead-in period day 15 pretreatment values of lipid profile parameters on post treatment days 30, 45 and 60 along with their respective percentage reductions have been presented in Tables 2-6.

P. glaucum bran at the rate of 6 gm/kg resulted in a significant lowering ($P<0.05$) of total cholesterol of liver in comparison to routine feed fed rabbits (Table 7). Moreover, it is also evident from the Table 7 that the total cholesterol lowering effect of 6 g/kg of *P. glaucum* bran and Simvastatin is non-significantly different.

As shown in Table 7, the level of cholic acid (mg/g) in the group of rabbits fed with 6 g/kg *P. glaucum* bran has been found significantly higher ($P<0.05$) than in those groups only on routine feed and simvastatin. Cholic acid is one of the constituents of bile prepared by cholesterol in the liver and necessary for emulsification of dietary fats making them suitable for on ward absorption from the intestine. Basically fiber forms a gel in the intestine that results in the binding of bile acids and secretion in feces. When an insufficient amount of cholesterol and bile acids reach the liver, the liver converts cholesterol to bile acids, thereby reducing cholesterol level in the serum (Patrick, 2002; Hossain *et al*., 2011). Serum cholesterol lowering

Table 1: Feeding schedule of normal routine rat feed, atherogenic feed, *P. glaucum* bran and Simvastatin in albino rats during the experimental period of 0 to 60 days.

Group	Group Detail	Treatment Plan	
		0-15 Days	16-60 Days
I	Untreated control on normal routine feed	Routine rat feed	
II	Untreated control on AF	AF (Routine rat feed + cholesterol 0.5%, coconut oil 20%, Cellulose 15%)	
III	Treated control on synthetic cholesterol lowering drug; Tablet survive® (simvastatin)	AF	AF+Tablets survive® (Simvastatin, 20 mg, 0.6 mg/kg) as cellulose replacement.
IV	*P. glaucum* bran	AF	AF+bran 2 g/kg BW as cellulose replacement.
V	*P. glaucum* bran	AF	AF+bran 4 g/kg BW as cellulose replacement.
VI	*P. glaucum* bran	AF	AF+bran 6 g/kg BW as cellulose replacement.

AF= Atherogenic feed BW= Body weight

Table 2: Values (Mean± SE) of total lipids (mg/dl) and their percentage reductions in the serum of hyperlipidemic rats (n=6) after treatment with *P. glaucum* bran and Simvastatin

Groups	Lead – in – Period Day 15	Post treatment days			Percentage reduction on post treatment days		
		30	45	60	30	45	60
I	225.99±2.9	224.49±2.45	219.82±2.39	216.2±2.26	-	-	-
II	391.29±3.1	439.05±3.1	512.44±2.6	579.43±2.5	-	-	-
III	373.78±3.9	297.69±2.8*	216.49±2.5*	201.81±2.2*	20.39±3.10	42.08±3.46	46.0±3.96
IV	405.45±2.7	384.41±2.8	360.69±2.6	355.81±2.1	5.18±3.2	11.03±3.41	12.24±4.12
V	375.68±3.3	338.80±2.9	306.77±2.6	271.72±1.9	9.81±.67	18.34±2.5	27.67±3.51
VI	366.26±2.4	310.02±2.6	257.61±2.8*	216.71±1.7*	15.36±3.27	29.66±3.76	@40.83±3.93

n=Number of animals in each group; *=significantly less (P≤0.05) than the pretreatment value at 15 days; @=Non-significantly (different from respective value obtained with Simvastatin.

Table 3: Values (Mean± SE) of triglycerides (mg/dl) and their percentage reductions in the serum of hyperlipidemic rats (n=6) after treatment with *P. glaucum* bran and Simvastatin.

Groups	Lead – in – Period Day 15	Post treatment days			Percentage reduction on post treatment days		
		30	45	60	30	45	60
I	62.38±2.88	61.52±2.5	63.09±2.40	60.72±2.29	-	-	-
II	127.66±1.81	142.40±1.9	163.52±1.6	194.0±1.1	-	-	-
III	126.84±1.45	95.97±2.08*	61.6±1.84*	52.52±1.52*	24.33±2.1	51.45±2.28	58.59±2.73
IV	132.39±2.69	121.47±2.48	111.27±2.41	101.12±2.06	9.24±1.47	15.95±1.71	23.61±2.47
V	124.51±1.73	111.12±2.3	101.37±2.04	91.72±1.3	10.75±1.95	18.58±2.07	26.33±2.13
VI	123.42±1.20	103.61±1.5	80.92±1.35*	62.98±1.02*	16.05±2.68	34.43±2.8	@47.97±3.17

n=Number of animals in each group; *=significantly less (P≤0.05) than the pretreatment value at 15 days; @=Non-significantly different from respective value obtained with Simvastatin.

Table 4: Values (Mean± SE) of total cholesterol (mg/dl) and their percentage reductions in the serum of hyperlipidemic rats (n=6) after treatment with *P. glaucum* bran and Simvastatin

Groups	Lead – in – Period Day 15	Post treatment days			Percentage reduction on post treatment days		
		30	45	60	30	45	60
I	88.04±3.10	87.63±2.76	88.20±2.51	86.47±2.36	-	-	-
II	144.44±1.3	162.62±2.87	190.81±2.60	212.05±2.40	-	-	-
III	141.40±2.79	109.04±2 .47*	83.09±1.48*	76.13±1.37*	22.17±2.08	41.23±2.45	48.28±3.01
IV	149.89±2.63	143.69±2.16	135.81±2.06	129.29±1.80	4.1±1.47	9.26±1.76	13.74±2.22
V	135.05±1.97	124.86±1.9	112.87±1.7	98.89±1.6	9.55±1.67	18.23±2.02	28.36±3.25
VI	133.75±2.58	113.52±2.10	96.53±1.8*	81.49±1.24*	15.12±3.15	27.82±3.68	@39.07±4.10

n=Number of animals in each group; *=significantly less (P≤0.05) than the pretreatment value at 15 days; @=Non-significantly different from respective value obtained with Simvastatin.

Table 5: Values (Mean± SE) of HDL- cholesterol (mg/dl) and their percentage increases in the serum of hyperlipidemic rats (n=6) after treatment with *P. glaucum* bran and Simvastatin

Groups	Lead – in – Period Day 15	Post treatment days			Percentage increase on post treatment days		
		30	45	60	30	45	60
I	31.95±2.98	31.58±2.69	31.79±2.45	31.37±2.31	-	-	-
II	25.74±1.93	22.90±1.71	21.58±1.30	19.09±1.10	-	-	-
III	23.265±1.79	30.68±1.89*	32.2±2.24*	34.79±2.91*	32.87±3.38	39.45±2.91	50.67±2.80
IV	23.99±1.60	24.99±1.69	26.58±1.99	28.30±2.20	4.16±3.30	10.79±2.92	13.79±2.25
V	26.91±1.61	28.40±1.91	29.77±2.56	30.29±2.80	5.5±2.02	10.62±1.80	15.18±1.60
VI	22.2±2.4	26.88±1.98*	30.18±2.81*	32.78±3.14*	21.08±1.75	35.94±1.56	@47.29±1.07

n=Number of animals in each group; * =significantly higher (P≤0.05) than the pretreatment value at 15 days; @=Non-significantly different from respective value obtained with Simvastatin.

Table 6: Values (Mean± SE) of LDL- cholesterol (mg/dl) and their percentage reductions in the serum of hyperlipidemic rats (n=6) after treatment with *P. glaucum* bran and Simvastatin

Groups	Lead – in – Period Day 15	Post treatment days			Percentage reduction on post treatment days		
		30	45	60	30	45	60
I	43.61±3.16	43.75±2.65	41.47±2.53	40.56±2.46	-	-	-
II	93.10±2.57	111.24±2.40	136.52±2.50	154.28±3.30	-	-	-
III	89.27±3.52	59.27±1.67*	39.59±1.51*	37.52±1.38*	33.60±2.14	55.65±2.25	57.97±3.30
IV	99.26±2.68	94.41±3.22	87.01±2.86	80.42±2.65	4.8±1.75	12.34±2.19	18.98±3.01
V	86.19±1.21	74.24±2.32	62.83±2.02	50.27±1.8	13.86±2.19	27.10±2.80	41.67±3.78
VI	86.87±2.05	66.01±1.86*	49.97±1.24*	39.24±1.05*	24.01±2.09	42.47±2.98	@54.82±3.12

n=Number of animals in each group; *=significantly less (P≤0.05) than the pretreatment value at 15 days; @=Non-significantly different from respective value obtained with Simvastatin.

Table 7: Total cholesterol (mg/g) in the liver and cholic acid (mg/g) in the feces of hyperlipidemic albino rats at post treatment day 60 after treatment with *P. glaucum* bran and Simvastatin

Group	Total cholesterol (mg/g)	Cholic acid (mg/g)
I	18.27±0.421B	80.2±3.6A
II	33.21±0.480C	89.7±4.5B
III	13.08±0.459A	78.1±3.2A
VI	15.71±0.497A	98.6±5.1C

Values (Mean±SE) followed by same letters indicate statistically non-significant difference (P>0.05).

effect of soyabean lecithin in rats (Polichetti *et al.*, 1996) and cellulose, Metamucil and pectin in man (Mukherjee, 2010) have been reported through promoting fecal elimination of cholesterol as bile acids.

In the light of above results *P. glaucum* seed bran was able to reduce significantly (P<0.05) the level of total lipids, triglycerides, total cholesterol and LDL-cholesterol in serum of hyperlipidemic albino rats at the dose rate of 6 g/kg feed, while serum HDL-cholesterol levels were increased. On post treatment day 60, the percentage reduction values of lipid profile and Simvastatin were found to be statistically similar (P<0.05). It can also be seen that *P. glaucum* barn at the dose of 6g/kg, amongst the lipid profile parameters reduced effectively LDL-cholesterol, triglycerides, total lipids and total cholesterol as 55, 48, 41 and 39%, respectively. Moreover, it is evident that *P. glaucum* bran at the dose of 6g/kg increased the level of HDL-cholesterol 47%. However, 40% reduction in serum total cholesterol was observed in hyperlipidemic rabbits fed with Cholestin, comprised of *Meniscus purpurea* rice and red yeast rice, while other lipid profile parameters remained statistically insignificant (Wei *et al*., 2003). On the other hand, *Commiphora mukul* as an adjunct to dietary therapy did not increase serum HDL-cholesterol in patients with hypercholesteremia (Singh *et al*., 1994). However, gemfibrozil and niacin have been found associated with significant increase in HDL-cholesterol by 24% and the ratio of total cholesterol to HDL-cholesterol decrease by 30% (Spencer *et al.*, 1996). Moreover, triglycerides were reduced by 22% in 70-80% patients treated with gugulipid (Nityanand *et al*., 1989).

A high fat diet is known to cause hyperlipidemia and there is a close relationship between atherosclerosis and an increase or decrease of serum lipids. In particular, LDL-cholesterol (bad cholesterol) may be risk factor and HDL-cholesterol (good cholesterol) may be a preventive factor. For the investigation of hypolipidemic activity, *P. glaucum* bran at dose rate 6g/kg and synthetic drug, Simvastatin, exhibited a significant lowering action on the serum levels of lipid profile parameters in albino rats. *P. glaucum* bran at dose rate 6 g/kg and Simvastatin induced respective percentage reductions in lipid profile parameters as 41 and 46% total lipids, 48 and 59% triglycerides, 39 and 48% total cholesterol and 55 and 58 % LDL-cholesterol. However *P. glaucum* bran at dose rate 6 g/kg and Simvastatin induced percentage increase in HDL-cholesterol as 47 and 51%, respectively. Direct relationship has been reported between coronary atherosclerosis and high blood levels of cholesterol, triglycerides and LDL-cholesterol (Anonymous, 1984). However, there is an inverse relationship between blood HDL-cholesterol level and coronary heart disease (Jahromi *et al*., 1993). After *P. glaucum* bran feeding, significant lowering of cholesterol (39%), triglycerides (47%) and LDL-cholesterol (55%) and increase in HDL-cholesterol (47%) may be taken as an indicative of risk reduction action. It is clear that *P. glaucum* bran at dose rate of 6 g/kg and Simvastatin 0.6mg/kg appear to have equal potency on the basis of increasing the level of HDL-cholesterol and decreasing the LDL-cholesterol. An increase in HDL-cholesterol and a reduction in LDL-cholesterol would be clinically lucrative as apo-B-containing lipoproteins are taken to be responsible for atherosclerosis (Choi *et al*., 1991). Thus, the clinical benefits of *P. glaucum* bran can be correlated with a fall in triglycerides, LDL-cholesterol and an increase in HDL-cholesterol. A clinically advantageous effect of *P. glaucum* bran at dose rate 6g/kg may also be related with the reduction of atherogenic index (total cholesterol/HDL-cholesterol) from 6.02 on lead-in day 15 to 2.49 on after treatment day 60. However, during this period Simvastatin reduced this ratio from 6.08 to 2.19.

The results of present study suggest that *P. glaucum* bran at dose rate 6 g/kg and Simvastatin 0.6mg/kg body weight were equally effective in the treatment of hyperlipidemic albino rats. Moreover, the potency of *P. glaucum* for stimulating fecal bile acid secretion in albino rats may safely be assumed, at least, as a part of mechanisms for its antihyperlipidemic efficacy. Further comprehensive chemical and pharmacological investigations are required to furnish the exact mechanism of these effects and also to isolate the active principle ingredients.

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