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Evaluation of hypolipidemic property of proso millet (*Panicum miliaceum* L.) in high fat diet induced hyperlipidemia in rats

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Abstract
This study was conducted to evaluate the impact of supplementation of proso millet based diet on high fat diet (HFD) induced hyperlipidemia in rats. A total of 48 numbers of healthy wister rats of either sex weighing between 120-200 g were selected for the study. After a period of acclimatization (1 week), the rats were randomly segregated into 8 different groups, each group having a total of 6 rats. Group I was as normal control fed a standard basal diet and Group II as treated control was fed a HFD containing 20% coconut oil. Group III, IV, and V were fed a HFD containing 10%, 20% and 40% whole grain (with husk) proso millet flour, respectively. Group VI, VII and VIII were fed HFD containing 10%, 20% and 40% dehusked (unpolished) proso millet flour, respectively. All animals had *ad libitum* access to food and water throughout the experiment. Results showed significant improvements in plasma high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, total cholesterol and triglycerides level in the groups supplemented with proso millets based diet as compared to the group fed HFD alone. Thus, it can be inferred that the proso millet is efficacious in lowering cholesterol levels in rats.

Keywords: Proso millet, hypolipidemic, hyperlipidemia, plasma lipid profile

1. Introduction
Hyperlipidemia is a major cause of concern because of the well established association between lipid concentrations and the risk of cardiovascular diseases (CVD), which is one of the leading causes of death throughout the world [1]. An elevated level of low-density lipoprotein cholesterol (LDL) is directly associated with development of atherosclerotic cardiovascular disease, which may present as coronary heart disease, stroke, and peripheral arterial disease [2]. Atherosclerosis is the result of hyperlipidemia and lipid oxidation and has always been a major cause of mortality in developed countries [3]. Approximately 52 million adults require lifestyle modifications including dietary changes and exercises, and 13 million adults need lipid-lowering medications to control their low density lipoprotein (LDL) levels [4]. Dietary factors that influence lipid levels include modification of nutritional components, consumption of specific foods, use of food additives and supplements, and major dietary approaches [5].

Millet is a generic term describing a range of small-seeded grains in two tribes Paniceae and Chlorideae of the family Poaceae [6]. Millets are commonly grown in most Asian and African countries, parts of Europe and consumed as a staple food among the majority of people of arid and semiarid tropics of the world [7]. Millets have nutraceutical properties in the form of antioxidants which prevent deterioration of human health such as lowering blood pressure, risk of heart disease, prevention of cancer and cardiovascular diseases, diabetes, decreasing tumor cases etc [8]. Proso millet (*Panicum miliaceum* L.) is the oldest cultivated millet crop and is often cultivated in harsh conditions as an alternative to maize because of their better adaptability to arid and barren lands than most other crops. It is superior to rice and wheat, because it provides protein, mineral and vitamins to the poor where the need for such nutrients is in high demand [9]. Proso millet is rich in nutraceuticals like dietary fibre, omega-3 fatty acids, phenolics, and flavonoids which play a potential role in treatment of degenerative diseases and metabolic disorder by improving cholesterol- metabolism, preventing the oxidative damage of body tissues and DNA as well as in the regulation of blood sugar [10, 11]. Phytochemicals from proso millets have increased interest due to their antioxidant, anti-microbial and anti-carcinogenic effects as well as other potential health benefits [12-14].

Proteins from proso millets also improve cholesterol metabolism [13]. Only recently has proso millet been promoted as a whole grain alternative in a healthy diet [15]. The food industry in Europe and North America is interested in proso millet for its potential health benefits for humans as well as its mild flavour, light colour and gluten free characteristics [16]. Therefore, in view of the various purported benefits of proso millet against a plethora of ailments affecting human health, the present study has been carried out to evaluate the efficacy of proso millet in lowering cholesterol levels in high fat diet (HFD) induced rats.

2. Materials and methods

2.1 Experimental animals

Adult healthy white albino rats (120 to 200 grams body weight) of either sex were procured from College of Veterinary Sciences, Department of Pharmacology and Toxicology, Assam Agricultural University, Khanapara, Guwahati-22. All the animals were housed in polypropylene cages in small groups of 6 rats. During the experiment, animals had free access to balanced food and clean drinking water *ad-libitum*, and were maintained in standard laboratory conditions (12:12 hour light/dark cycle at ambient temperature ranged between 25 °C to 28 °C).

The use of experimental animals and the study protocol was duly approved by the Institutional Animal Ethics Committee (IAEC) of the college.

2.2 Animal diets

The normal control diet consisted of 100% M/s Hindustan lever rat ration (protein 23%, fat 5%, crude fibre 5%, ash 10%, calcium 2%, phosphorus 1%, and corn starch 54%). A high fat diet (HFD) was made by supplementing 20% coconut oil to the above normal diet substituting the same quantity of rat ration to create hyperlipidemia [17]. The test diets were prepared by replacing rat ration with powdered proso millet, both whole grains (with husk) and dehusked (unpolished) at three dietary levels (10%, 20% and 40%) respectively, into the HFD. All diets were formulated to contain approximately 20% protein and 6% fat. Diets were stored at 4 °C in air tight containers.

2.3 Experimental design for animal experiment

A total of 48 (forty eight) rats were taken for the study. After a period of *acclimatization* (1 week), the rats were randomly divided into 8 (eight) diet groups, each of 6 (six) rats. Group I received normal standard diet (100% rat ration), and served as normal control. Group II served as treated control and received high fat diet (HFD). Animals in Group III, IV, and V were fed with HFD containing 10%, 20% and 40% whole grain (with husk) proso millet flour, respectively. Rest of the animals in Group VI, VII and VIII were fed on HFD containing 10%, 20% and 40% dehusked (unpolished) proso millet flour, respectively. All groups were fed their corresponding diets for 4 weeks (28 days). All animals had *ad libitum* access to food and water throughout the experiment. Food intakes were recorded daily and body weights were recorded at weekly intervals.

Table 1: Composition of different experimental diets

Group	Number of animals	Composition of diet
I	6	Normal control: 100% RR
II	6	Treated control (HFD): 80% RR + 20% CO
III	6	70% RR + 10% WGPMF + 20% CO
IV	6	60% RR + 20% WGPMF + 20% CO
V	6	40% RR + 40% WGPMF + 20% CO
VI	6	70% RR + 10% DPMF + 20% CO
VII	6	60% RR + 20% DPMF + 20% CO
VIII	6	40% RR + 40% DPMF + 20% CO

RC: Rat ration; CO: Coconut oil; HFD: High- fat diet; WGPMF: Whole grain proso millet flour; DPMF: Dehusked proso millet flour

2.4 Collection of blood

After an overnight fast blood samples were withdrawn separately in heparinized sterile centrifuge tubes by puncture from the inner retro-orbital plexus of the eye from all rats every week on 0^{th}, 7^{th}, 15^{th}, 21^{st} and 28^{th} days of experimentation and centrifuged at 3000 rpm for 15 minutes to obtain plasma. The blood samples were then subjected to biochemical investigation for the determination of plasma lipid profile.

2.5 Estimation of plasma lipid profile

Parameters like high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), total cholesterol and triglycerides were estimated spectrophotometrically in different experimental groups of the animals using standard commercial kits (DIATEK).

2.6 Statistical analysis

The results of animal experiment were expressed as the Mean ± standard deviation (SD).

3. Results and Discussion

3.1 Impact of supplementation of proso millet on plasma high density lipoprotein cholesterol (HDL) level (mg/dl) in rats

Impact of supplementation of proso millet on plasma HDL level (mg/dl) in experimental rats is depicted in Table 2. The results were indicated that the HDL levels decreased towards the last week in group II (12.32 mg/dl) that was fed high fat diet alone as compared to group V (22.45 mg/dl) fed 40% whole grain proso millet and group VIII (21.08 mg/dl) fed 40% dehusked proso millet where a significant increase in the HDL levels was observed. The increase in the HDL levels in group V may be attributed to the anti- oxidant [18] and free radical scavenging property [19] inherent in proso millet. Studies have shown that anti-oxidants are effective in raising the HDL levels in a variety of subjects [20]. The presented study is in accordance to the finding of previous workers where it was found that the feeding of millet elevated plasma HDL cholesterol levels in rats and mice by stimulating the synthesis of HDL in liver or small intestine and raise the plasma concentration as the result [13, 21, 22].

Table 2: Impact of supplementation of standard basal diet, whole grain proso millet and dehusked proso millet on plasma HDL level (mg/dl) in experimental rats

Groups	0 day	7 days	15 days	21 days	28 days
Group I	16.44±0.71	16.89±0.38	16.72±0.25	16.65±0.37	16.53±0.57
Group II	16.20±0.58	15.76±0.42	14.62±0.35	13.76±1.06	12.32±0.25
Group III	17.31±0.54	17.60±0.45	17.62±0.15	17.76±0.28	18.77±0.51
Group IV	15.97±0.76	16.43±0.66	16.79±0.53	17.91±0.44	19.91±0.91
Group V	16.34±0.73	17.26±0.79	18.16±0.47	20.22±0.34	22.45±0.78
Group VI	17.27±0.53	17.38±0.37	17.46±0.46	17.59±0.29	17.71±0.28
Group VII	15.97±0.76	16.25±0.38	16.53±0.36	16.70±0.29	17.29±0.35
Group VIII	17.22±0.33	17.68±0.42	18.08±0.47	19.09±0.37	21.08±0.50

3.2 Impact of supplementation of proso millet on plasma low density lipoprotein cholesterol (LDL) level (mg/dl) in rats

The results of Impact of supplementation of proso millet on plasma low density lipoprotein cholesterol (LDL) level (mg/dl) in rats are presented in Table 3. Results revealed that there was significant increase in the LDL level towards the last week in high fat diet supplemented group II (102.06 mg/dl) as compared to the 40% whole grain proso millet supplemented group V (78.92 mg/dl) and 40% dehusked proso millet supplemented group VIII (90.92 mg/dl). The decrease in the LDL level in group V and VIII may be attributed to the changes that are brought about at the genetic level such as up-regulated expression of adiponectin and down-regulated tumor necrosis factor alpha (TNF-alpha) [22]. Previous study reported that the down regulation of tumor necrosis factor alpha (TNF-alpha) has led to the lowering of LDL levels in blood [23]. Presented study is in harmony to the finding of previous workers Thatola *et al.* [10], where it was concluded that supplementation of foxtail millet biscuits produced a significant reduction from baseline value by 20% for serum LDL-cholesterol because millet have a higher proportion of non-starchy polysaccharides and dietary fibre. The improvement in LDL-cholesterol level seen in the present study may be due to the similar mechanism as proso millet is rich source of dietary fibres such as water soluble gum and β-glucans.

Table 3: Impact of supplementation of standard basal diet, whole grain proso millet and dehusked proso millet on plasma LDL level (mg/dl) in experimental rats

Groups	0 day	7 days	14 days	21 days	28 days
Group I	45.88±0.80	46.49±0.92	45.20±0.95	45.21±0.95	44.84±0.72
Group II	46.04±0.53	57.08±1.24	79.32±1.34	91.63±2.51	102.06±1.25
Group III	45.58±0.88	54.24±0.48	74.09±0.74	81.69±1.09	90.66±1.52
Group IV	45.27±0.97	53.04±0.33	70.94±0.82	77.17±1.22	86.21±1.04
Group V	45.22±0.55	49.96±0.77	66.40±1.01	72.78±0.95	78.92±0.90
Group VI	46.52±1.07	56.53±1.10	78.01±0.87	88.93±1.20	97.57±1.33
Group VII	45.29±1.24	54.33±1.28	75.21±1.18	86.10±1.73	93.49±1.68
Group VIII	45.75±0.91	52.88±0.62	72.76±1.00	82.88±1.37	90.92±1.36

3.3 Impact of supplementation of proso millet on plasma total cholesterol level (mg/dl) in rats

Impact of supplementation of proso millet on plasma total cholesterol level (mg/dl) in experimental rats is shown in Table 4. The results indicated that the total cholesterol levels were significantly decreased in the whole grain proso millet supplemented groups and dehusked proso millet supplemented groups as compared to the high fat diet supplemented group. The decrease in plasma total cholesterol could be due to presence of beneficial dietary fibre and phytochemicals with potent antioxidant activity in millets [24]. Earlier studies have shown that chlorogenic acid, a phenolic acid, is very effective in lowering cholesterol levels [25]. Chlorogenic acid is abundantly present in the proso millets [26]. There, the decline in the cholesterol level may be chalked up to the presence of chlorogenic acid in proso millets. The present findings were in conformity with reports of Thatola *et al.* [10], found that supplementation of foxtail millet biscuits produced a significant reduction from baseline value by 6% for serum total cholesterol.

Table 4: Supplementation of standard basal diet, whole grain proso millet, dehusked proso millet on plasma total cholesterol level (mg/dl) in experimental rats

Groups	0 days	7 days	14 days	21 days	28 days
Group I	75.20±0.97	75.53±0.89	74.95±0.74	75.11±0.44	74.56±0.77
Group II	75.25±0.86	87.40±0.94	124.42±1.53	142.67±1.48	163.75±1.80
Group III	75.30±0.38	84.91±0.44	117.37±1.32	134.44±1.29	153.34±1.60
Group IV	75.84±0.75	83.27±0.42	115.34±0.81	130.26±0.70	148.93±0.96
Group V	75.55±0.95	80.71±0.58	107.74±0.53	123.22±2.25	142.70±1.77
Group VI	74.61±0.63	86.45±0.75	121.90±1.20	138.98±2.03	159.71±0.83
Group VII	75.61±0.54	86.29±0.84	112.08±0.88	138.05±0.72	157.42±0.67
Group VIII	75.80±0.44	85.62±0.73	120.62±0.70	135.85±0.92	154.47±1.34

3.4 Impact of supplementation of proso millet on plasma triglycerides level (mg/dl) in rats

Impact of supplementation of proso millet on plasma triglycerides level (mg/dl) in experimental rats is given in Table 5. Tetriglycerides levels were significantly elevated in the high fat diet group (HFD) as compared to the whole grain proso millet and dehusked proso millet supplemented groups. Earlier studies reflected a pattern resembling the present study

where supplementation of proso millet has led to a significant decline in the serum triglyceride levels in controlled subjects [27]. The present finding was consistent with the study of Thatola *et al.* [10], revealed that supplementation of foxtail millet biscuits produced a slight yet non- significant decrease of triglycerides, because of the presence of higher proportion of non-starchy polysaccharides and dietary fibre.

Table 5: Impact of supplementation of standard basal diet, whole grain proso millet, dehusked proso millet on plasma triglycerides level (mg/dl) in experimental rats

Groups	0 days	7 days	14 days	21 days	28 days
Group I	94.49±1.67	92.68±1.73	93.11±1.69	93.41±1.64	93.60±1.64
Group II	92.16±0.69	92.42±0.68	92.69±0.68	92.94±0.69	93.21±0.69
Group III	96.92±0.78	95.80±0.87	94.43±0.79	93.86±0.76	92.89±0.78
Group IV	71.52±1.23	70.50±1.31	67.73±1.17	64.81±1.28	62.88±1.27
Group V	84.67±1.94	82.53±1.70	77.29±1.95	74.60±1.92	70.14±1.96
Group VI	74.94±1.51	74.47±1.52	73.87±1.51	73.04±1.51	72.05±1.53
Group VII	95.12±1.41	93.89±1.46	91.99±1.42	90.53±1.32	89.34±1.40
Group VIII	65.86±1.34	64.69±1.37	61.50±1.39	60.01±1.38	57.91±1.21

4. Conclusion

The present study revealed that proso millet consumption can significantly improve the plasma lipid profiles in experimental rats and its adequate supplementation in the diet can drastically bring down the instances of hyperlipidemia. Present study confirmed and extended the understanding that proso millet may beneficially affect the hyperlipidemia and attribute to positive alteration in the plasma lipid level. Thus, proso millet could provide protection against the onset of cardio-vascular disease. Therefore, the outcome of the present research can be used as valuable information for emphasizing the significance of proso millet as a functional food ingredient to treat hyperlipidemia or reduce the risk of atherosclerosis and thus improving overall health of the population.

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