Research Article

Anti-Hyperglycemic Effect of Cereal and Millet Based Modified Starch Substituted Pasta Foods

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Abstract

The combinations of whole grain cereals and millets based modified starch substituted low glycemic functional pasta products were formulated to improve the hypoglycaemic effect. The present investigation was undertaken to assess the effect of cereal and millet based modified starch substituted noodles on changes in body weight, plasma glucose, haemoglobin and glycosylated haemoglobin and lipid profile. Based on animal experiments, the T_4 and T_7 samples fed group (G6 and G9) delivered slightly higher weight gain when compared to other experimental group and in normal diet fed group. The reduction percentage of blood glucose level in diabetic rats at 28 days of duration was observed to be 39.0 to 44.5 per cent (experimental group G4 to G9) and comparatively slight higher reduction was noticed in T_4 (G6) and T_7 (G9) samples. Except G2 group (diabetic rats untreated group) the plasma insulin values for all the groups were found to be higher indicating that the treatments had a positive effect on insulin levels. Glycosylated Hb of the treatment groups in (G3-G9) was statistically lower than the diabetic control group, though more than that of normal control rats. The experimental group showed significant reduction in the cholesterol level at the end of the 28 days of the study.

Keywords: Cereals and millets, modified starch, pasta products, hypoglycaemic effect

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Introduction

The incidence of diabetes mellitus is increasing in an exponential manner globally. According to recent estimates, approximately 285 million people worldwide in the 20 - 79 year age group are affected with diabetes in 2010, 438 million people of the adult population, is expected to have diabetes by 2030 [1]. Calling India the 'Diabetes capital' of the world, the International Journal of Diabetes in developing Countries says that there is an alarming rise in. For India, this increase is estimated to be from 51 million people in 2010 to 87 million in 2030 [2].

Cereals form a major portion of human diet and are an important source of starch and other dietary carbohydrates (dietary fiber), which play an important role in the energy requirement and nutrient intake of human. Whole-grain consumption is a part of a healthy diet described as the 'prudent' diet and is rich source of phytochemicals which offer several health benefits [3]. Epidemiological studies consistently show that the risk for type 2 *diabetes mellitus* is decreased with the consumption of whole grains [4, 5]. Millet ranks as the sixth most important grain in the world, sustains 1/3 of the world's population.

Extrusion cooking, as a multi-step, multi-functional and thermal process, is implied in the innovation of functional low glycemic functional food products [6]. Extrusion cooking is used worldwide for the production of expanded snack foods, modified starches, ready to eat cereals, baby foods, pasta and pet foods. This technology has distinct advantages like versatility, low cost, better product quality and no process effluents [7].

It is well recognized that diet plays a major role in controlling type 2 diabetes. The evidence through many studies suggests that millets and resistant starch (retrograded starch) appears to be an attractive source of dietary fiber that have potential benefits to mitigate or delay the onset of complications associated with diabetes and lower the serum cholesterol concentration. Hence, this present study was undertaken to ascertain the scientific basis for the use of

millets and modified starch (retrograded resistant starch fraction which acts as an analogous to dietary fiber) utilized in the development of pasta products in the management of diabetes using alloxan - induced diabetic Wistar albino rats.

Methodology

Wistar albino rats each weighing 180 - 220 g was obtained from Thiruvananthapuram Medical College, Thiruvananthapuram, India and the study was conducted. The experiments were approved by the Institutional of Animal Ethics Committee, India. The animals were housed in large spacious cages and they were fed with commercial pellets and access to water *ad libitum*. The animals were well acclimatized to the standard environmental condition of temperature (22°C ± 5°C) and humidity (55 ± 5%) and 12 hrs light dark cycles throughout the experimental period.

Diabetes mellitus was induced in Wistar rats by single intraperitoneal injection of freshly prepared solution of Alloxan monohydrate (150 mg/kg BW) in physiological saline after overnight fasting for 12 hrs [8]. Alloxan is commonly used to produce diabetes mellitus in experimental animals due to its ability to destroy the β-cells of pancreas possibly by generating the excess reactive oxygen species such as H_2O_2, O_2 and HO^-. The development of hyperglycemias in rats is confirmed by plasma glucose estimation 72 hrs post alloxan injection. The rats with fasting plasma glucose level of >150 mg/dL were used for this experiment.

In the experiment, a total of 54 rats (48 diabetic surviving rats and six normal rats) were used. Diabetes was induced in rats three days before starting the experiment. The rats were divided into eight groups after the induction of 150 mg/kg of alloxan monohydrate through I.P. diabetes.

Treatment Protocol

- Group - I: (Normal control) consist of normal rats given with 10 ml/kg of normal saline with normal diet.
- Group - II: (Diabetic control) Diabetic control received normal diet.
- Group - III: (Treatment control group) Diabetic rat received whole wheat flour noodle ($_{T1}$) for 28 days.
- Group - IV: (Treatment group) Diabetic rat received whole wheat flour + kodo millet flour noodle (T_2) for 28 days.
- Group - V: (Treatment group) Diabetic rat received whole wheat flour + kodo millet flour + cassava modified starch noodle (T_3) for 28 days.
- Group - VI: (Treatment group) Diabetic rat received whole wheat flour + kodo millet flour + cassava modified starch + green gram dhal flour noodle (T_4) for 28 days.
- Group - VII: (Treatment group) Diabetic rat received whole wheat flour + barnyard millet flour noodle (T_5) for 28 days.
- Group - VIII: (Treatment group) Diabetic rat received whole wheat flour + barnyard millet flour + cassava modified starch noodle (T_6) for 28 days.
- Group - IX: (Treatment group) Diabetic rat received whole wheat flour + barnyard millet flour + cassava modified starch + green gram dhal flour noodle (T_7) for 28 days.

After 28 days of treatment, the blood glucose level and body weight was measured. The blood was collected in fresh vials containing EDTA as anticoagulant agents and plasma was separated in a T8 electric centrifuge at 2000 rpm for 2 minute. The serum samples were used for various biochemical procedures [9].

Biochemical Analysis

Blood glucose was estimated by commercially available glucose kit based on glucose oxidase method [10]. Plasma insulin was determined by ELISA method using a Boehringer-Mannheim kit [11] with an ES300 Boehringer analyzer (Mannheim, Germany). Total haemoglobin was determined by the method of [12] and glycosylated haemoglobin was determined by the method of [13]. Plasma lipids were determined by auto analyzer according to the method of [14].

Statistical Analysis

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA) and the group means were compared by Newman-Keuls multiple range test (NKMRT).

Results and Discussion

The results obtained by conducting animal experiments on alloxan - induced diabetic albino rats by feeding therapeutic noodles (T_1 to T_7-Group III - IX) and compared with the normal and diabetic control rats (Group 1 & II) for 28 days are tabulated and are presented in the **Table 1**.

Table 1 Effect of functional pasta product (noodles) on body weight of normal and alloxan - induced albino rats

Groups	Initial Body Weight (Gram)	Final Body Weight (Gram)
GROUP I (G 1)	205.12±3.60	218.65± 4.20
GROUP II (G 2)	175.52±5.40	164.43± 2.40*[a]
GROUP III (G 3) T_1	174.42±4.20	204.00±5.13*[b]
GROUP IV (G 4) T_2	171.21±3.34	214.86± 4.13*[b]
GROUP V (G 5) T_3	170.54±5.62	213.17± 5.13*[b]
GROUP VI (G 6) T_4	172.64±5.27	220.11± 3.13*[b]
GROUP VII (G 7) T_5	173.2±4.75	218.23± 3.42*[b]
GROUP VIII (G 8) T_6	172.20±4.64	214.21± 4.52*[b]
GROUP IX (G 9) T_7	170.24± 3.75	217.90± 5.42*[b]

As expected, an increase in blood glucose and cholesterol was observed in the alloxan - induced rats. From the Table 1 the data on the change in body weight of normal and experimental rats was noticed. Feeding rats with functional pasta product (cooked noodles) for 28 days significantly reversed the body weight loss of diabetic rats to near normal level. A weight reduction was observed in diabetic control rats. The percentage increase in body weight in diabetic treated rats with the functional pasta product (noodles) was found to be significantly higher than that in the normal control and in diabetic control animals due to the maintenance of optimum protein level and also reduction in glucose levels. In fact, the incorporation of modified starch yielded reductions in the feed intake due its effect on satiety, generated lower percentage increase in weight gain in G5(T_3) and G8(T6) (24.9 and 24.4%)when compared to other treatment group except treatment control group (G3). The T_4 and T_7 samples fed group G6 and G9 delivered slightly higher weight gain percentage as 27.5 and 28.0 per cent increase when compared to other experimental group and in normal diet fed group.

The blood protein levels were maintained in the functional pasta product-fed rats (millet and modified starch) especially in T_4 and T_7 samples due to its higher protein content than other experimental samples. The reduction in protein in the diabetic rats and its increase on feeding the functional noodles corresponded well with the body weight changes.

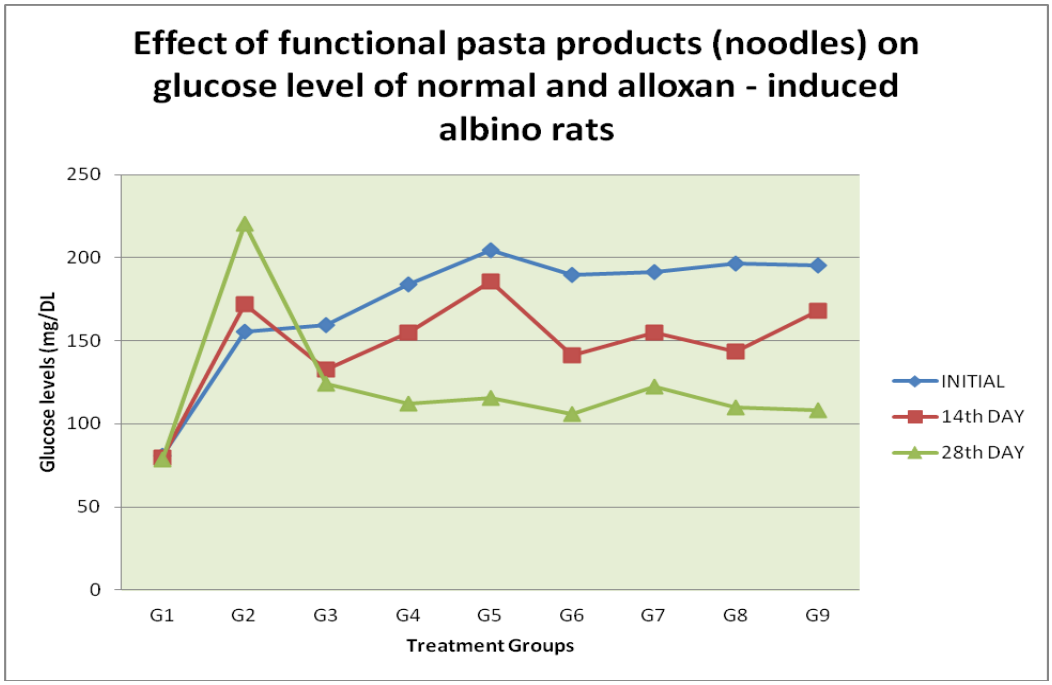
It was observed that feeding the functional pasta product to rats reduced the blood glucose level after 28 days of the study period (**Table 2**).

Table 2 Effect of functional pasta products (noodles) on glucose level of normal and alloxan - induced albino rats

Groups	Initial	14th Day	28th Day
GROUP I (G 1)	80.55±3.12	79.60±3.50	78.45±4.50
GROUP II (G 2)	155.70±3.75	172.30±8.20**[(a)]	220.53±7.43**[(a)]
GROUP III (G 3) T_1	159.42±3.95	132.52±4.48**[(b)]	124.34±4.57**[(b)]
GROUP IV (G 4) T_2	183.78±3.58	154.72± 4.05**[(b)]	112.10±3.57**[(b
GROUP V (G 5) T_3	204.78±3.60	185.76±3.90**[(b)]	115.70±4.37**[(b)]
GROUP VI (G 6) T_4	189.50±3.75	141.32±3.92**[(b)]	106.12±4.16**[(b)]
GROUP VII (G 7) T_5	191.42±3.85	154.64±3.42**[(b)]	122.47±4.74**[(b)]
GROUP VIII (G 8) T_6	196.33± 4.45	143.44±2.62**[(b)]	109.94±3.37**[(b)]
GROUP IX (G 9) T_7	195.12±3.45	168.24±3.48**[(b)]	108.29±4.40**[(b)]

There was a significant lowering of blood sugar level in rats fed diets in all combinations from T_1 to T_7. The dietary fiber content of kodo / barnyard millet and the modified starch (resistant starch acts as dietary fiber) contributes to the hypoglycemic effect on diabetic rats. The mechanism of action for glucose reduction in diabetic patient is similar to that of other soluble fibers, because it forms a viscous gel in aqueous solution, it may slow the access of glucose to the small intestine's absorptive epithelium, thereby blunting postprandial glucose peaks. Soluble

fibers may delay gastric emptying, slowing carbohydrate uptake. A third mechanism that may contribute to the postprandial effect of carbohydrates ingested with the meal, retarding carbohydrate access to digestive enzymes.



The retardation of glucose diffusion is also due to the inhibition of α-amylase, thereby limiting the release of glucose from the starch. The inhibition of α-amylase activity by medicinal plants might be attributed to several possible factors such as fiber concentration, the presence of inhibitors on fibers, encapsulation of starch and enzyme by the fibers present in the sample, thereby reducing accessibility of starch to the enzyme and direct adsorption of the enzyme on fibers, leading to decreased amylase activity. Inhibitors of carbohydrate hydrolyzing enzyme delay carbohydrate digestion and prolong overall carbohydrate digestion time, causing a reduction in the rate of glucose adsorption and consequently blunting the postprandial plasma glucose rise. The reduction percentage of blood glucose level in diabetic functional pasta product fed rats at 28 days of storage was observed to be 39.0 to 44.5 per cent and comparatively slight higher reduction was noticed in T_4 (G6) and T_7 (G9) samples. The initial glucose level of G6 and G9 treatment group was found to be 189.50 ± 3.75and 195.12 ± 3.45and reduced to 106.12± 4.16 and 108.29± 4.40 mg/dL respectively (44.0 and 44.5 %). The protein content and its network of the samples and especially in T_4 and T_7 samples as through the green gram dhal flour contributed significant amounts is very important in inhibiting starch digestion because it encapsulates starch granules, and thus restricts accessibility of alpha amylase. Both the dietary fiber and protein network of T_4 and T_7 samples fed in G6 and G9 rats contributed in hypoglycemic effect.

The plasma insulin, haemoglobin and glycosylated haemoglobin were assessed after the feeding trial and are tabulated in the **Table 3**.

Table 3 Effect of functional pasta product (noodles) on haematological parameters of normal and alloxan - induced albino rats

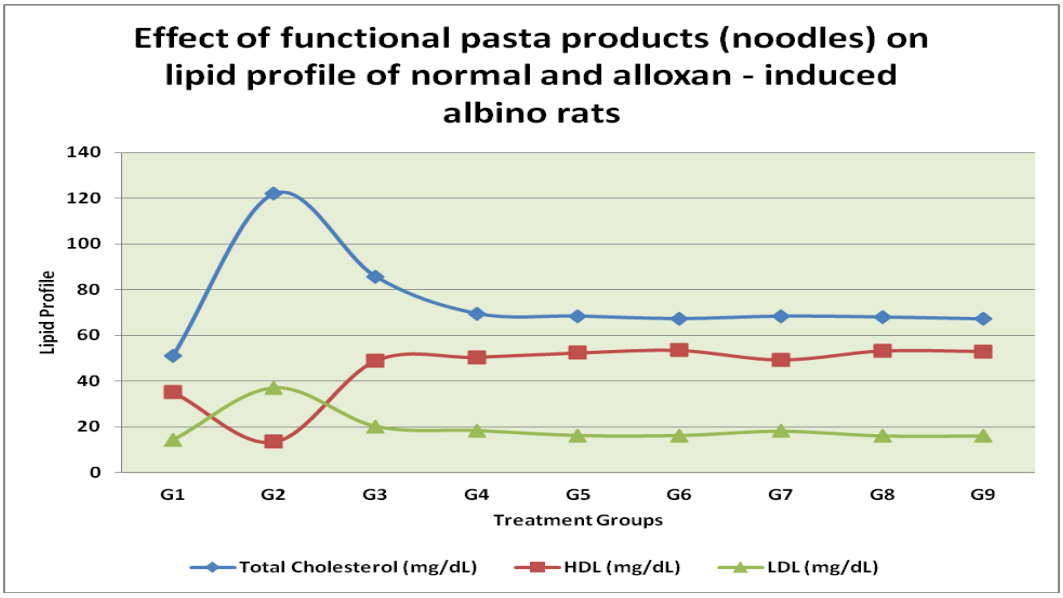
Group	Plasma Insulin(µU/ml)	Haemoglobin (mg/dL)	Glycosylated haemoglobin (%)
GROUP I (G 1)	25.30±1.26	0.30 ±0.06	30.30±0.75
GROUP II (G 2)	6.20±0.60**$^{(a)}$	0.92 ±0.12**$^{(a)}$	12.60±0.22**$^{(a)}$
GROUP III (G 3)T_1	11.68±0.88**$^{(b)}$	0.40 ±0.06**$^{(b)}$	21.50±0.62**$^{(b)}$
GROUP IV (G 4)T_2	16.15±0.92**$^{(b)}$	0.35 ±0.07**$^{(b)}$	25.30±0.70**$^{(b)}$
GROUP V (G 5)T_3	21.65±0.96**$^{(b)}$	0.33 ±0.05**$^{(b)}$	26.88±0.75**$^{(b)}$
GROUP VI (G 6)T_4	22.40±0.78**$^{(b)}$	0.30 ±0.05**$^{(b)}$	26.01±0.65**$^{(b)}$
GROUP VII (G 7)T_5	16.85±0.80**$^{(b)}$	0.36 ±0.08**$^{(b)}$	26.55±0.60**$^{(b)}$
GROUP VIII (G8)T_6	20.88±0.88**$^{(b)}$	0.34 ±0.04**$^{(b)}$	27.05±0.80**$^{(b)}$
GROUP IX (G 9)T_7	22.00±0.59**$^{(b)}$	0.31 ±0.04**$^{(b)}$	27.96±0.75**$^{(b)}$

The plasma insulin level in normal control rats which consisted was 25.30±1.26 µu/ml. Plasma insulin level of G5, G6, G8 and G9 were close to that of G1. The G2 rats which were diabetic induced, and with no treatment given, had the least plasma insulin of 6.20± 0.60 µu /ml. Except G2 group the plasma insulin values for all the groups were found to be higher indicating that the treatments had a positive effect on insulin levels. Glycosylated haemoglobin of the treatment groups in (G3-G9) was statistically lower than the diabetic control group, though more than that of normal control rats. Presence of kodo / barnyard millet, modified starch and green gram dhal appears to have an appreciable hypoglycemic effect on the diabetic rats. In poorly controlled or uncontrolled diabetes there is an increased glycosylation of a number of proteins including haemoglobinnn and α-crystalline of lens [15]. Glycosylated haemoglobin was found to increase in patients with diabetes to approximately 16 per cent [16] and the amount of increase in diercly proportional to the fasting blood glucose level [17]. During diabetes the excess glucose presentin blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in alloxan induced rats [18]. Administration of noodles for 28 days prevents a significant elevation in glycosylated haemoglobin there by increasing the level of total haemoglobin in diabetic rats. This could be due to the result of improved glycemic control produced by noodles.

The effect of the functional pasta products on the lipid profile of the normal (G1), toxic control (G2) and treatment groups (G3-G9) are presented in the **Table 4**.

Table 4 Effect of functional pasta product (noodles) on lipid profile of normal and alloxan - induced albino rats

GROUPS	Total Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	Phospholipids (mg/dL)	LDL (mg/dL)
GROUP I (G 1)	50.95±2.45	63.45±2.30	35.20±1.70	115.60±2.35	14.30±1.32
GROUP II (G 2)	122.15±6.75**(a)	162.85±4.60**(a)	13.40±1.25**(a)	218.60±6.25**(a)	36.90±2.26**(a)
GROUP III (G 3)T_1	85.80±2.60**(b)	90.75±2.48**(b)	48.88±1.45**(b)	130.40±2.95**(b)	20.18±1.80**(b)
GROUP IV (G 4)T_2	69.55±2.30**(b)	86.65±2.85**(b)	50.45±1.46**(b)	121.55±2.72**(b)	18.35±1.92**(b)
GROUP V (G 5)T_3	68.45±2.36**(b)	85.30±2.60**(b)	52.35±1.55**(b)	118.45±2.86**(b)	16.22±1.68**(b)
GROUP VI (G 6)T_4	67.32±2.64**(b)	84.45±2.22**(b)	53.43±1.40**(b)	117.36±2.90**(b)	16.16±1.78**(b)
GROUP VII (G 7) T_5	68.40±2.78**(b)	86.05±2.89**(b)	49.30±1.35**(b)	122.65±2.86**(b)	18.05±1.95**(b)
GROUP VIII (G 8)T_6	68.02±2.44**(b)	85.00±2.45**(b)	53.22±1.62**(b)	119.50±2.56**(b)	16.01±1.65**(b)
GROUP IX (G 9)T_7	67.21±1.25**(b)	85.00±1.07**(b)	53.00±0.66**(b)	117.14±0.25*(b)	16.00±0.74**(b)



The experimental group showed significant reduction in the cholesterol level at the end of the 28 days of the study. The total cholesterol, triglyceride, phospholipids and LDL levels were lower in G6 and G9 group when compared to other groups and HDL levels were found to be slightly higher in G6 and G9 group. In the alloxan induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in serum cholesterol and triglycerides. The levels of cholesterol and triglycerides and low density lipoprotein levels were brought to near

normal by the treatment with noodles in alloxan induced diabetic rats. The main anti-antherrogenic lipoprotein is involved in the transport of cholesterol from peripheral tissues into liver and thereby it acts as a protective factor against coronary heart disease [19].

Therefore, the animal experiment results proved that the development of enriched pasta with a higher dietary fibre content (kodo/barnyard millet and modified starch (improved resistant starch content) would be a good way to increase the fiber intake and reduce the glycemic index of pasta, which would result in a product for specific nutritional purposes.

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