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RESEARCH ARTICLE

Antidiabetic action of prebiotic convenient food mixes: An *in vivo* rat study

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Abstract

Diabetes is a chronic multi-system disease having long term health consequences. This study investigated the role of multi millet enriched prebiotic convenient food mixes in alleviating the diabetic complications in induced diabetic rats. The multi millets and leafy vegetables *viz.,* drumstick leaves (*Moringa oleifera*) and spinach leaves (*Spinacia oleracea*) were used to formulate convenient food mixes namely chappathi and dosa mix as well as evaluated the antidiabetic effect of these mixes in alloxan induced diabetic albino rats. All the developed four mixes are scored higher acceptability and provided with high amounts of fibre, iron and beta carotene. The study comprises 6 groups of 6 animals each. Group 1 served as non-diabetic control, Group 2 as diabetic control and groups 3, 4, 5 and 6 served as treatment groups. Diabetic was induced in rats by administration of alloxan monohydrate (150 mg/kg) through the intra peritoneal route. Treatment groups (G3 - G6) received diet of (100 mg/kg of body weight) multi millets drumstick leaves chappathi mix (T1); multi millet spinach leaves chappathi mix (T2); multi millet drumstick dosa mix (T3) and multi millet spinach leaves dosa mix (T4) respectively. After the 28 days of treatment, diabetic rats showed a highly significant ($p>0.0001$) reduction in blood glucose levels when compared to diabetic control and non-diabetic groups. The study showed the multi millets and leafy vegetable based instant mixes have an effective antidiabetic property which controls the blood glucose levels in the rats and this study will create a new avenue to formulate the special convenient food mixes for diabetic people.

Keywords: antidiabetic; convenient food mixes; millets; prebiotic food

Introduction

Cereal based foods contribute more than fifty percent of the global food produced and they are grown in over 73 % of the world (*1*). Increasing urbanization and mecha- nization in most countries has led to a sedentary lifestyle and a diet having high fat foods with highly refined patterns are contributing to several chronic degenerative diseases such as diabetes mellitus, cardiovascular diseases and cancer. Diabetes mellitus is a silent disease and is now recognized as one of the fastest growing threats to public health in almost all countries of the world. According to the World health organization reports, India an approximate of 77 million people above the age of 18 years are suffering from diabetes and nearly 25 million are prediabetic stage. More than 50 % of people are unaware of their diabetic status which leads to other health complications. (2). Millet contributes to the prevention of diabetes since its having the properties of slow releases glucose into the blood for a longer duration than commonly consumed other cereals. Millets support in body weight control, which is crucial for diabetic management. Further millet increases insulin sensitivity and reduces triglyceride levels in the body (3). India is a leading producer of small millet *viz*., foxtail millet, little millet, kodo millet, proso millet and finger millet. The cultivation of millet is more in Madhya Pradesh, Chattisgarh, Orissa, Tamil Nadu, Jharkhand, Karnataka, Andhra Pradesh and Maharashtra (4). The hypoglycemic effect of minor millets with their high crude fibre, antioxidant, low carbohydrate content, low digestibility and presence of β-glucans which are water soluble gums is helpful in repairing glucose metabolism (5). These grains release sugar slowly in the blood and diminish glucose absorption. Higher intake of fruit, especially green leafy vegetables, yellow vegetables, cruciferous vegetables or their fiber is associated with a lower risk of type 2 diabetes. Increasing consumption of fruit and vegetables may be the primary prevention of many chronic diseases (6). *Moringa oleifera* is an Indian tree that has been cultivated in diverse regions referred to as "drumstick tree". The leaves are eaten as vegetables of food ingredients because of the high content of vitamins, antioxidants and macronutrients to improve nutritional deficiencies (7). Similarly, spinach (*Spinacia Oleracea*) or spinach leaves are a dietary vegetable that ranks high among other vegetables in terms of antioxidant capacity and an excellent source of vitamin K, vitamin A, E, folate and many other nutrients like inositol, choline, omega 3 fatty acids, selenium and niacin. It is rich in glucuronides and therefore has anti proliferative, anti-inflammatory and antimutagenic properties (8). Convenience foods are defined as items that require little or no preparation. Convenience foods are available in many ways. Breakfast is the most important meal of the day and breakfast cereals offer the most nutrient dense and lowest fat choice at breakfast time. They are convenience foods made from processed grains, which need very little time for cooking (9). Millets have great potential for being utilized in different food systems by virtue of their nutritional quality and economic importance. There is a wide scope of their exploitation in different food products including bakery products, instant mixes and convenience food mixes etc. Hence, the present study focused on development and evaluation of multi millets and leafy vegetables-based convenience food mixes and the products were analyzed for proximate composition, sensory attributes and their antidiabetic active vegetables-based the Wistar albino rats.

Materials and methods

Collection of the samples

The millets such as kodo millet, foxtail millet, barnyard millet and little millet were procured from the Department of Millets, Tamil Nadu Agricultural University, Coimbatore and dehulled by using double chamber centrifugal dehuller, cleaned, washed and sundried, milled into flour. The remaining ingredients were procured from local departmental stores.

Preparation of composite flour

Multi millet composite flour blends were prepared using millet flour *viz.,* kodo millet flour (25 %), little millet flour (25 %), foxtail millet flour (25 %), barnyard millet flour (25 %).

Formulation of convenience food mixes

The various treatments of whole wheat flour with combinations of multi millet composite flour and dried drumstick leaves (*Moringa oleifera*) / dried spinach leaves (*Spinacia oleracea*) was carried out in various proportions to formulated low glycemic chappathi mix. Similarly, multi millet composite flour with combinations dried drumstick leaves/ dried spinach leaves with other minor ingredients in various proportions to formulate low glycemic dosa mixes also carried out. Among the different proportions best combination were selected for the further studies the selected combinations of mixes are given in Table 1.

Organoleptic evaluation

The sensory evaluation of multi millet drumstick leaves chappathi, multi millets spinach leaves chappathi, multi millet drumstick dosa and multi millet spinach leaves dosa mix made from formulated convenience food mixes were performed by 30 panellists. The panellists asked to evaluate color, appearance, taste, flavor, texture and overall acceptability. The ratings were on a 9-point hedonic scale, ranging from 9 as like extremely to 1 as dislike extremely (10). Sensory trials were replicated thrice.

Nutrient analysis

Developed multi millet mixes were estimated for carbohydrates and crude fibre by the method (11). Crude protein (Micro kjeldahal, Nx6.25), crude fat (solvent extraction), calcium (titration), iron and beta carotene (colorimetric) were determined by the AOAC (12) and for energy was calculated by nutritive value of Indian food (13).

Pharmacological study

Animal collection

Antidiabetic activity study of millet product was conducted in Wistar albino rats. The rats weighted around 200-300 g were taken and placed at random and allocated to treatment groups in poly propylene cages and paddy husk as bedding. Animal care procedure and experimental protocol were approved by the institutional Animal Ethics Committee (KU/IAEC/153/2016-17), Karpagam University, Coimbatore, 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 ± 5° C) and humidity (55 ± 5 %) and 12 hr light dark cycles throughout the experimental period.

Induction of diabetes

Diabetes mellitus was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared alloxan monohydrate solution (150 mg/kg of body weight) in physiological saline after overnight fasting for 12 hr. Alloxan is commonly used to induce 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 hr post alloxan injection. The rats with fasting plasma glucose level of 160-220 mg/dl were used for this experiment.

Table 1. Proportion of ingredients in the formulated mixes.

Mixes	Multi millet composite flour (g)	Wheat flour (g)	Black gram flour (g)	Fenugreek seeds flour (g)	Dried drumstick leaves (g)	Dried spinach leaves (g)	Cumin seeds (g)	Chilli powder (g)	Dried curry leaves (g)	Salt (g)
T_1	49	49	-	-	2	-	-	-	-	2
T_2	49	49	-	-	-	2	-	-	-	2
T_3	65	-	23	2		2	1	1	1	2
T_4	65	-	23	2	2	-	1	1	1	2

T_1- Multi millet drumstick leaves chappathi mix; **T_2**- Multi millets spinach leaves chappathi mix; **T_3**- Multi millet drumstick dosa mix and **T_4**- Multi millet spinach leaves dosa mix.

Animal grouping and treatment

A total of 36 rats (30 diabetic surviving rats and six normal rats) were used for the study. The rats were divided into 6 groups of 6 each. Diabetes was induced in rats of 5 groups by injecting 150 mg/kg of alloxan monohydrate intra-peritoneally 3 days before starting the experiment. The details of the group division are as follows.

Group 1: (Normal control) consisted of normal rats given 10 ml/kg of normal saline with normal diet.

Group 2:(Diabetic control) rats received 150 mg/kg of Alloxan monohydrate through I.P. injection.

Group 3: (Treatment control group) Diabetic rats received multi millet drumstick leaves chappathi mix (T_1) at a dose of (100 mg/kg orally) for 28 days.

Group 4: (Treatment group) Diabetic rats received multi millet spinach leaves chappathi mix (T_2) at a dose of (100 mg/kg orally) for 28 days.

Group 5: (Treatment group) Diabetic rats received multi millet drumstick dosa mix (T_3) at a dose of (100 mg/kg orally) for 28 days.

Group 6: (Treatment group) Diabetic rats received multi millet spinach dosa mix (T_4) at a dose of (100 mg/kg orally) for 28 days.

Sample collection

The levels of blood glucose were measured at the starting day and after 10^{th}, 20^{th} and 28^{th} days of feeding trial. The blood samples were collected retro-orbitally from the eye under light ether anesthesia using capillary tubes in fresh vials containing EDTA (Ethylene Di-amine Tetra Acetic Acid) as anticoagulant agent and serum was separated in a T8 electric centrifuge at 2000 rpm for two min. Then serum samples were used for blood glucose test (14).

Statistical analysis

The experiments were conducted in triplicates and the data were expressed as Mean ± Standard Deviation (S.D). For animal experiments, all the values were expressed as Mean ± SEM. The data was analyzed using analysis of variance (ANOVA) and the values were considered statistically significant at $p<0.0001$.

Results and Discussions

Organoleptic evaluation

Subjective sensory characteristics multi millet and leafy vegetable based convenient food mixes are summarized in Table 2. All the developed four mixes scored higher acceptability and within the mixes, multi millet spinach leaves chappathi mix (8.7 ± 0.14) had higher overall acceptability followed by multi millet drumstick leaves chappathi mix (8.6 ± 0.16), multi millet drumstick leaves dosa mix (8.6 ± 0.22) and multi millet spinach leaves dosa mix (8.4 ± 0.20). Green leafy vegetable contain chlorophyll content imposed the dark greenish colour in the products and this was affected the colour score of organoleptic evaluation. Similarly (15) to develop multi millet vegetable roti mix by using multi-grain flour of foxtail millet, kodo millet, little millet and barnyard millet with rice flour and dehydrated vegetables. The result revealed that vegetable roti prepared by incorporating 70 % multi millet flour obtained highest acceptability on sensory evaluation. The traditional fermented food *idli* from small millets with black gram dhal and found that *idli* prepared with little millet, kodo millet and barnyard millet were had higher acceptability in previous studies (16).

Nutrient composition

The results of nutrient composition of convenient mixes are presented in Table 3. The protein and crude fibre content were found to be high in all the four mixes since the multi millets have high amount of fibre and due to the incorporation of drumstick leaves and spinach leaves. Among the mixes, protein content was higher in multi millet spinach leaves chappathi mix (13.20 ± 0.32) by other mixes and the values ranged from 10.18 ± 0.29 to 12.70 ± 0.38 % respectively. Preparation of a mix of composite flours of foxtail, barnyard and finger millet with wheat flour by adding 10 - 30 % millet flour and observed addition of milled millet flour to wheat flour increased the concentration of protein and fat but decreased the carbohydrates in past research investigation (17). With respect to fibre content multi millet drumstick leaves dosa (10.12 ± 0.60) than the other mixes. High crude fibre performs the role on lower glycemic index and lipidemic responses and adds to health benefits by providing faecal bulk matter, reducing

Table 2. Sensory score of multi millet based convenient mixes.

Mixes	Colour	Flavour	Texture	Taste	Overall acceptability
T_1	8.2±0.17	8.4±0.16	8.6±0.10	8.4±0.50	8.6±0.16
T_2	8.1±0.10	8.6±0.12	8.7±0.04	8.7±0.02	8.7±0.14
T_3	8.2±0.30	8.7±0.18	8.5±0.28	8.6±0.18	8.6±0.22
T_4	8.1±0.14	8.4±0.16	8.5±0.20	8.5±0.16	8.4±0.20

T_1- Multi millet drumstick leaves chappathi mix; **T_2**- Multi millets spinach leaves chappathi mix; **T_3**- Multi millet drumstick dosa mix and **T_4**- Multi millet spinach leaves dosa mix.

Table 3. Sensory score of multi millet based convenient mixes.

Mixes	Carbohydrate (g)	Protein (g)	Fat (g)	Crude fibre (g)	Calcium (mg)	Iron (mg)	Beta carotene (µg)	Energy (Kcal)
T_1	64.07 ± 2.34	10.08± 0.29	2.36± 0.76	4.64± 0.10	41.76± 1.45	4.21 ± 0.45	125.57 ± 3.12	316
T_2	54.32 ± 2.56	13.20± 0.32	2.14± 0.82	4.53± 0.54	54.75 ± 2.76	3.64 ± 0.53	106.86 ± 2.15	302
T_3	60.54 ± 3.04	11.99± 0.45	2.22 ±0.88	10.12± 0.60	132.60 ± 4.21	4.07 ± 0.36	215.03 ± 3.54	309
T_4	58.59 ± 2.68	12.70 ± 0.38	2.67 ±0.57	5.67 ± 0.78	77.39 ± 2.43	3.87 ± 0.24	191.97 ± 3.42	309

Values are expressed as mean ± SEM.

intestinal transit time, preventing constipation and in turn providing protection against colorectal cancer (3). The formulated mix by little millet (51.0 %), dried coconut (32.8 %) and sugar (16.2 %) and this mix provide 5.05 - 6.53 g of protein, 140 mg of calcium and 5.96 - 7.72 mg of iron per 100 g of sample. The green leafy vegetable *viz.*, drumstick leaves and spinach leave provide the beta carotene to the mixes. The highest content of beta carotene found in Multi millets drumstick dosa mix (215.03 ± 3.54) while least content found in multi millet spinach left chappathi mix (106.86 ± 2.15). reported that Addition of pumpkin seeds, carrot and cowpea leaves that are rich in beta carotene content in the millet based composite flour enhanced the vitamin A and iron content in previous studies (18).

Antidiabetic activity

Various aspects of animal studies and human nutrition intervention trials proved that millet foods are known to be hypoglycemic because of high fibre content. The antidiabetic effect of the multi millet mixes along with leafy vegetables by conducting experiments on Wistar albino rats and the results are presented in Table 4.

The initial fasting blood glucose level of normal rats was 94.12 ± 15.34 and diabetic induced rats ranged between 151.17 ± 14.56 to 169.67 ± 12.86. On the first day of feeding trials normal rats had blood glucose level 95.44 ± 14.32 mg/dl (G1) while that of the diabetic group was 313.00 ± 12.56mg/dl (G2). At the end of 28 days feeding trial, fasting blood glucose level was significantly increased to 332.83 ± 14.54 mg/dl in diabetic rats as compared to normal rats (95.88 ± 14.89 mg/dl). However, the level of fasting blood glucose returned to near normal range in the experimental groups (G3 - G7). The blood glucose level gradually decreased trends in multi millet mix fed (G3 - G6) ranged between 106.80 ± 14.20 to 116.33 ± 12.44 whereas constant increased level seen in diabetic control (G2). Among the mixes the rat fed with T4 diet (multi millet spinach leaves dosa mix) had higher rate of reduction followed by T_1, T_3 and T_2 diet. The presence of high fibre from millets and leafy vegetables contributed to the hypoglycemic effect on rats. The overall result indicated that the reduction in the blood glucose levels was high in the rats fed with multi millet mixes. Since millet and leafy vegetable-based mixes contained high amounts of dietary fibre and beta carotene, these mixes release sugar slowly in the blood and diminish the glucose absorption. The effect of insoluble dietary fibre in the inhibition of glucose diffusion in the small intestine is suggested to be due to the absorption or inclusion of the smaller sugar molecules within the structure of the fibre particles. The result is supported in previous research investigations by replacing rice-based dosa with foxtail millet-based dosa showed a significant reduction in the postprandial blood glucose levels (19). The barnyard millet khichdi had very slow release of blood sugar, a quality suited and desirable for diabetic patients (20). Biscuits prepared by substituting 50 % of refined wheat flour with barnyard millet flour had a lower glycemic index, GI (50.17) compared to the GI of wheat biscuits (73.58) without much difference in the nutrient composition (21). Similarly, earlier scientific studies (22) revealed that spinach leaves khakra supplemented for 60 days showed good glucose reducing effect and HBA1C values in the post prandial. The dietary strategies aim at improving both diabetes control and cardiovascular risk factors is the use of low-glycemic index diets. Diet has been the sheet anchor in the management of diabetes. The efficacy of multi-millet mix consisting of foxtail millet, barnyard millet, soyabean, flaxseeds, groundnut, bengal gram dhal in reducing blood glucose level in type II diabetic patients was studied in earlier research (23). Their fasting and postprandial blood glucose levels indicated a significant decrease in the level of both diabetic (160.2 and 150.2) and non-diabetic (92.21 and 89.6) subjects. The studies assessed the effect of millet-based diet in 300 patients with type 2 diabetes mellitus for 90 days and compared the effects of the millet-based diet on glycemic control and plasma lipid concentrations. The millet-based diet lowered HbA1c (19.14 %), fasting glucose (13.5 %), insulin (1.9 %) concentrations, total cholesterol concentrations (13.25 %), triglyceride concentrations (13.51 %) and very-low density lipoprotein cholesterol concentrations by 4.5 % in the patients with type 2 diabetes (24).

Conclusion

Our study results have shown that multi millets enriched prebiotic convenient foods mixes exert remarkable antidiabetic action in alloxan-induced diabetic rats. Enhancing the antidiabetic potential of the formulated convenient foods was remarkable in comparison to control food mixes. This type of dietary consumption would be a significant way to increase the fibre intake and reduce the glycemic index. Processing and utilization of millets in product development have promising prospects about nutrition, quality and health benefits and can be an alternative to cereals.

Table 4. Antidiabetic effect of multi millet mixes in albino rats.

Treatment	Days intervals Fasting blood glucose level (100 mg/dl)				
	Initial day	1st	10th	20th	28th
G 1	94.12±15.34	95.44±14.32	95.47±13.42	96.67±14.65	95.88±14.89
G 2	153.67±14.56	313.00±12.56	342.50±14.89	364.67±13.59	332.83±14.54 ** (a)
G 3	152.50±12.10	316.00±20.32	194.17±13.85	113.50±12.34	110.00±15.43 ** (b)
G 4	169.67±12.86	344.50±16.70	232.33±12.45	137.00±13.53	116.33±12.44 ** (b)
G 5	154.83±13.50	352.13±18.43	178.17±12.40	132.53±12.44	113.54±13.56 ** (b)
G 6	151.17±14.56	304.17±11.25	214.50±16.45	179.17 ±16.43	106.80±14.20 ** (b)

G1- Control group; **G 2**- Diabetic Control; **G 3** - Treatment group (T1); **G 4** - Treatment group (T2); **G 5**- Treatment group (T3) ; **G 6** - Treatment group (T4). Values are expressed as mean ±SEM. ** (**a**) Significantly different from normal control G1 at P<0.01, ** (**b**) Significantly different from Diabetic control G2 at P<0.01.

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

Declaration of generative AI and AI-assisted technologies in the writing process

Author(s) hereby declares that generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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