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Research Article

Efficacy of Value Added Multi Millet Convenient Foods in the Management of Diabetes in Albino Rats

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Abstract

In India, eight millets species (Sorghum, Pearl millet, Finger millet, Foxtail millet, Kodo millet, Proso millet, Barnyard millet and Little millet) are commonly cultivated under rain fed conditions. The diet based on millets and leafy vegetables having antidiabetic properties gives a new approach in the treatment of diabetic mellitus. The study was carried out to formulate multi millet based convenient chappathi and dosa mix with incorporation of dried drumstick and palak leaves and evaluated the anti diabetic effect of these mixes in alloxan induced diabetic albino rats. All the developed four mixes are scored higher acceptability and provided high amount of fibre, iron and beta carotene. In the anti diabetic study, rats were divided into 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 (150mg/kg) through the intra peritoneal route. Treatment groups (G3-G6) received diet of (100mg/kg of body weight) multi millets drumstick leaves chappathi mix (T_1); multi millet palak leaves chappathi mix (T_2); multi millet drumstick dosa mix (T_3) and multi millet palak leaves dosa mix (T_4) respectively. After the 28 days of treatment, diabetic rats showed highly significant reduction in blood glucose levels when compared to diabetic control and non diabetic groups. Hence the study concluded that multi millets and leafy vegetable based instant mixes have an effective antidiabetic property which controlling the blood glucose levels in the rats and also this study will create a new avenue to formulate the special convenient food mixes for diabetic people.

Keywords: Small Millets; High Fibre; Convenient Food Mixes; Anti Diabetic Activity

Introduction

An increasing urbanisation, westernization and mechanization in most countries has led to a sedentary lifestyle and a diet having high energy foods 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 Apollo diabetics group studies and researches proved that consumption of rice coupled with sedentary lifestyles and lack of exercise increases the likelihood of type 2 diabetes multi fold in south India. It was found that people consuming around 400 grams of rice are in the very high risk groups. [1]. Global prevalence of diabetes is escalating in an exponential manner. An estimated 382 million people worldwide were affected by diabetes in 2013, and this number is expected to rise to 592 million by 2035. Consequently, diabetes is predicted to become the major cause of death and disability in the world by 2030 [2]. 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 [3]. 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 [4]. These grains release sugar slowly in the blood and also 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 [5]. Moringa oleifera is an Indian tree that has been cultivated in diverse regions referred to as “drum stick tree”. The leaves are eaten as vegetables of food ingredient because of the high content of vitamins, antioxidants and macronutrients to improve nutritional deficiencies [6]. Similarly the Spinach (Spinacia oleracea) or palak 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 [7]. Convenience foods are defined as item that requires little or no preparation. Convenience foods are available in many forms. 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 needs very little time for cooking. [8] 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 develop and evaluation of multi millets and leafy vegetables based convenience food mixes and the products were analysed for proximate composition, sensory attributes and their antidiabetic activity evaluated in 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 was 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/dried palak leaves was carried out in various proportions to formulated low glycemic chappathi mix. Similarly multi millet composite flour with combinations dried drumstick leaves/dried palak 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 is given in table 1.

Organoleptic evaluation

The sensory evaluation of multi millet drumstick leaves chappathi, multi millets palak leaves chappathi, multi millet drumstick dosa and multi millet palak leaves dosa mix made from formulated convenience food mixes were performed by 30 panelists. 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 [9]. Sensory trials were replicated thrice.

Mixes	Multi millet composite flour (g)	Wheat flour (g)	Black gram flour (g)	Fenugreek seeds flour (g)	Dried drumstick leaves (g)	Dried palak 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

Table 1: Proportion of ingredients in the formulated mixes.

T_1: Multi millet drumstick leaves chappathi mix; T_2: Multi millets palak leaves chappathi mix; T_3: Multi millet drumstick dosa mix and T_4: Multi millet palak leaves dosa mix.

Nutrient analysis

Developed multi millet mixes were estimated for carbohydrates and crude fibre by the method given by [10]. Crude protein (Micro kjeldahal, Nx6.25), crude fat (solvent extraction), calcium (titration), iron and beta carotene (colorimetric) were determined by the AOAC [11] and for energy was calculated by Nutritive value of Indian food [12].

Pharmacological study

Animal collection

Antidiabetic activity study of millet product was conducted in wistar albino rats. The rats weighted around 200-300g 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 hrs. 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 hrs post alloxan injection. The rats with fasting plasma glucose level of 160-220mg/dl were used for this experiment.

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 intraperitoneally 3 days before starting the experiment. The details of 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 150mg/kg of Alloxan monohydrate through I.P. injection.
- **Group 3:** (Treatment control group) Diabetic rats received multi millet drumstick leaves chappathi mix (T1) at a dose of (100 mg/kg orally) for 28 days.
- Group 4: (Treatment group) Diabetic rats received multi millet palak leaves chappathi mix (T2) at a dose of (100 mg/kg orally) for 28 days.
- Group 5: (Treatment group) Diabetic rats received multi millet drumstick dosa mix (T3) at a dose of (100 mg/kg orally) for 28 days.
- Group 6: (Treatment group) Diabetic rats received multi millet palak dosa mix (T4) at a dose of (100 mg/kg orally) for 28 days.

Sample collection

The levels of blood glucose, was measured at the starting day and after 10th, 20th and 28th days of feeding trial. The blood samples were collected retro-orbitally from the eye under light ether anaesthesia using capillary tubes in fresh vials containing EDTA (Ethylene Di-amine Tetra Acetic Acid) as anticoagulant agent and serum was separated in a T_8 electric centrifuge at 2000 rpm for two minutes. Then serum samples were used for blood glucose test [13].

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 $p<0.01$.

Results and Discussion

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 palak leaves chappathi mix (8.7 ± 0.14) had score higher over all 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 palak 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 [14] to develop Multi millet vegetable roti mix by using multi-grain flours 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 per cent multi millet flour obtained highest acceptability on sensory evaluation. [15] evaluated 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.

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

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

T_1: Multi millet drumstick leaves chappathi mix; T_2: Multi millets palak leaves chappathi mix; T_3: Multi millet drumstick dosa mix and T_4: Multi millet palak leaves dosa mix.

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 having high amount of fibre and also due to the incorporation of drumstick leaves and palak leaves. Among the mixes, protein content was higher in multi millet palak leaves chappathi mix (13.20 ± 0.32) by other mixes and the values ranged from 10.18 ± 0.29 to 12.70 ± 0.38 per cent respectively. Singh., *et al.* [16] prepared composite flours of foxtail, barnyard and finger millet with wheat flour by adding 10-30% millet flour and observed that addition of milled millet flour to wheat flour increased the concentration of protein and fat but decreased the carbohydrates. With respect to fibre content multi millet drumstick leaves dosa (10.12 ± 0.60) than the other mixes. High crude fibre perform the role on lower glycemic index and lipidemic responses and adds to health benefits by providing

faecal bulk matter, reducing 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 provides 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 palak leaves 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 palak leaved chappathi mix (106.86 ± 2.15). Tumwine [17] reported that pumpkin seeds, carrot and cowpea leaves are rich in beta carotene content which included in the millet based composite flour enhanced the vitamin A and iron content.

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

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

Values are expressed as mean **±** SEM.

Anti diabetic activity

Various aspects of animal studies and human nutrition intervention trials proved that millet foods are known to be hypoglycaemic because of high fibre content. 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 the normal rats was 94.12 ± 15.34 and diabetic induced rats were 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

Treatment	Days Intervals Fasting blood glucose level (100 mg/dl)				
	Initial day	1st	10th	20th	28th
G1	94.12 ± 15.34	95.44 ± 14.32	95.47 ± 13.42	96.67 ± 14.65	95.88 ± 14.89
G2	153.67 ± 14.56	313.00 ± 12.56	342.50 ± 14.89	364.67 ± 13.59	332.83 ± 14.54 ** (a)
G3	152.50 ± 12.10	316.00 ± 20.32	194.17 ± 13.85	113.50 ± 12.34	110.00 ± 15.43 ** (b)
G4	169.67 ± 12.86	344.50 ± 16.70	232.33 ± 12.45	137.00 ± 13.53	116.33 ± 12.44 ** (b)
G5	154.83 ± 13.50	352.13 ± 18.43	178.17 ± 12.40	132.53 ± 12.44	113.54 ± 13.56 ** (b)
G6	151.17 ± 14.56	304.17 ± 11.25	214.50 ± 16.45	179.17 ± 16.43	106.80 ± 14.20 ** (b)

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

G1: Control group; G2: Diabetic Control; G3: Treatment group (T_1); G4: Treatment group (T_2); G5: Treatment group (T_3); G6: Treatment group (T_4).

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

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 was gradually decreased trends in multi millets mix fed (G3-G6) ranged between 106.80 ± 14.20 to 116.33 ± 12.44 where as constant increased level seen in diabetic control (G2). Among the mixes the rat fed with T4 diet (multi millet palak leaves dosa mix) had higher rate of reduction followed by T1, T3 and T2 diet Presence of high fibre from millets and leafy vegetables contributed the hypoglycaemic effect in rats. The overall result indicated that the reduction in the blood glucose levels was high in the rats fed with multi millet mixes. Since the millet and leafy vegetable based mixes contained high amounts of dietary fibre and beta carotene, these mixes release sugar slowly in the blood and also 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 supported by Narayanan [18] replacing rice-based dosa with foxtail millet-based dosa showed a significant reduction in the postprandial blood glucose levels. The barnyard millet khichdi had very slow release of blood sugar, a quality suited and desirable for diabetic patients [19]. Biscuits prepared by substituting 50% of refined wheat flour with barnyard millet flour had lower glycemic index, GI (50.17) compared to the GI of wheat biscuits (73.58) without much difference in the nutrient composition [20]. Similarly Karthikaa and Usha [21] studies 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. Meti [22] studied 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. 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 studied 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 percent in the patients with type 2 diabetes [23].

Conclusion

From the findings of this study, it may be concluded that the alloxan-induced diabetes mellitus caused an increase in blood glucose and also feeding of millet and leafy vegetable based diets have antidiabetic effects. 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 with regard to nutrition, quality and health benefits and can be an alternative to cereals.

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