Foods 2021

MDPI

Proceedings

Roasted Whole-Grain Barnyard Millet Flour Effective in Reversing Metabolic Signs of Diet-Induced Metabolic Syndrome Rats †

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† Presented at the 2nd International Electronic Conference on Foods, 15–30 October 2021; Available online: https://foods2021.sciforum.net/.

Abstract: Millet is recognized as one of the beneficial cereals that can provide adequate nutrients and several studies have demonstrated that supplementation of millet has potential to possess valuable effects on the lifestyle disorder. However, the possible benefits of barnyard millet to the metabolic disorder disease are very limited. New insight in this area is important to improve the variety of healthy diet to the consumer, especially to the individual with metabolic syndrome. Thus, this study aimed to determine the effect of roasted whole-grain barnyard millet flour on the diet-induced metabolic syndrome rats. We investigated the reversal sign of the metabolic syndrome of male Wistar rats on 10% addition of roasted whole-grain barnyard millet flour to modified high carbohydrate high fats diet and corn starch diet for the final eight weeks of a 16-week protocol. The rats were divided into five groups (n = 7) and each of the groups received a special diet for 16 weeks: normal rats' pallet (N), corn starch diet with barnyard millet (CM), corn starch diet (CS), high-carbohydrate high-fats diet with barnyard millet (HM) and high-carbohydrate high-fats diet (HF). After 16 weeks of diet regime, the intervention rats' group HM had a significant reduction ($p < 0.05$) in area under the curves of blood glucose level between week 8 and week 16, and showed a significant difference ($p < 0.05$) in weight, abdominal circumference, fat mass and systolic blood pressure with HF group. The results demonstrated the important potential of roasted whole-grain barnyard millet health-promoting effect, specifically in reversing metabolic syndrome disease.

Keywords: barnyard millet; obesity; dyslipidemia; hypertension; high carbohydrate diet; high fat diet

Citation: Mohd, M.S.; Shafie, S.R.; Shafie, N.H.; Ahmad, F. Roasted Whole-Grain Barnyard Millet Flour Effective in Reversing Metabolic Signs of Diet-Induced Metabolic Syndrome Rats. *Proceedings* **2021**, *1*, x. https://doi.org/10.3390/xxxxx

Published: 15 October 2021

1. Introduction

Metabolic syndrome (MetS) is entitled as a cluster of metabolic abnormalities that directly increase the risk of having cardiovascular disease and type II diabetes [1]. The risk factors of MetS include central obesity, elevated blood pressure, impaired glucose tolerance, insulin resistance and low high-density lipoprotein (HDL) cholesterol [2,3]. The presence of three out of these five criteria considered an individual diagnosed with MetS [4]. The prevalence of MetS escalated globally and it was contributed by the sedentary lifestyle and unhealthy dietary pattern. Nowadays, food high in carbohydrates, particularly simple sugar and high in fats become a regular diet, especially in a developing country.

Including plant-based functional foods, especially cereals as a part of healthy dietary regimes, can help maintain good health. Millet is a type of cereal majorly consumed in the

African and Asian region, rich in phytochemicals and nutrients. These gluten-free cereals are getting much attention nowadays and reported to be a good choice for people with celiac disease [5] and also beneficial to human health. Barnyard millet is a variety of millet that contains excellent carbohydrates, protein, dietary fiber and most remarkably contains micronutrients, especially iron and zinc, compared to other major cereals like rice, wheat, and maize [6].

Existing evidence showed that barnyard millet contains slowly digestible carbohydrates, attributed from its high carbohydrate to crude fiber ratio, which enables the slow release of sugar in the blood and consequently can help in maintaining the blood sugar level [6]. Besides, polyphenols from barnyard millet show anti-hyperglycemic activity by inhibiting α-glucosidase which can improve glucose tolerance [7]. In addition, daily oral administration of barnyard millet to type II diabetic mice model showed lower results of hepatic lipid parameters, including reduced total cholesterol and triglyceride compared to the diabetic control group [8]. These previous findings showed that barnyard millet has the potential in treating metabolic disorders. However, there is still no study on the health benefits of introducing barnyard millet to diet-induced metabolic syndrome. A reproducible animal model of MetS to investigate the potential benefits of barnyard millet is vital to understand the complexity of its potentiality.

Hence, this study aimed to determine the effect of adding 10% roasted barnyard millet in a daily diet of high carbohydrate, high-fat diet-induced rats' model to mimic the human metabolic syndrome. In this study, we investigate the reversible effect of metabolic syndrome by assessing the rats' cardiovascular condition (blood pressure) and metabolic responses (body weight, abdominal circumference, body composition and glucose tolerance). We induced rats with a modified high carbohydrate, high-fat (HCHF) diet where the fat source comes from ghee, a usual animal fat used in Southeast East Asian diet. This is to ensure the HCHF diet for metabolic syndrome rats' model imitates the pathogenesis of MetS in South East Asians, which is currently at an increasing state for this condition caused by rapid lifestyle changes. New insight into the functional food of millet could improve the variety of healthy diet to the humankind especially to the individual with metabolic syndrome.

2. Materials and Method

2.1. Millet preparation

Whole grain barnyard millet were purchased originally from India. The millet was clean thoroughly to remove any foreign materials. Then, the whole grain barnyard millet were roasted at 110 °C for 10 min in the convection oven. The roasted seed were cooled and ground into a fine powder using a heavy-duty blender. The flour was passed through the sieved and stored at 4 °C for future use.

2.2. Animal Experimental Protocol

All animal experiments were reviewed and approved by Universiti Putra Malaysia Institutional Animal Care and Use Committee (UPMIACUC/AUP-R0072020). 8–9-week male Wistar rats weighing between 200-250g were purchased from Takrif Bistari Enterprise, Selangor, Malaysia. The rats were housed individually under standard temperature (27 °C) and alternate 12h light/dark cycle in the animal house facility of Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. Upon the arrival, the rats were acclimatized for two weeks and were given ad libitum of water and standard rats food pellet.

The rats were randomly divided into five experimental groups (n = 7/group): normal pellet (N), corn starch diet (CS), corn starch diet with 10% roasted whole grain barnyard millet (CM), high carbohydrate high-fat diet-fed rats (HF), and high carbohydrate high-fat diet with 10% roasted whole grain barnyard millet (HM). The normal group was fed with standard rat pallet (Gold Coin, Selangor, Malaysia). The CS and CM diet were fed with cornstarch diet suggested from the previous study [3] containing 57% cornstarch,

15.5% powdered rat food, 2.5% Hubble, Mendel and Wakeman salt mixture and 25% water. The CS group were fed with cornstarch diet for 16 weeks while the CM diet were fed with corn starch for 8 weeks and 10% of millet were added in the diet at the remaining 8 week of experiment protocol. The HF and HM group were fed with diet proposed by [9] containing 17.5% fructose, 39.5% sweetened condensed milk, 20% ghee, 15.5% powdered rat food, 2.5% Hubble, Mendel and Wakeman salt mixture. HF group were fed for 16 weeks and the HM group were fed with high carbohydrate and high-fat for 8 week and the remaining 8 weeks of experiment protocol, 10% of millet were added to their diet. N, CS and CM groups were given a clean tap water while HF and HM group were given water supplemented with 25% fructose. All food and drinks were given ad libitum. Food and drinks intake were monitored and recorded daily.

2.3. Measurements of the Rats

The weight of the rats was recorded daily. Abdominal circumferences were measured after light anesthesia. Systolic blood pressure of the rats was accessed using noninvasive tail cuff system (CODA™, Kent Scientific Corporation, Torrington, CT, USA) after warming the rats for 10 min.

Oral glucose tolerance test (OGTT) was performed on 8th week and 16th week. The test was performed after overnight fasting using blood from tail vein with FreeStyle Optium Neo glucometer (Abbott Laboratories, Bedford, MA, USA). OGTT were conducted where the rats were given a glucose load of 2g/kg (body weight) as 40% glucose solution via oral gavage. Blood glucose concentrations were measured again at 30, 60, 90 and 120 min after glucose administration.

Body compositions of the rats were measured using dual-energy X-ray absorptiometry (DEXA) (Hologic Qdr-100 System) at week 16 (Hologic QDR-1000 System) under anesthesia of ketamine-xylazine via intraperitoneal injection. DEXA scans were analyzed using manufacturer recommended software (Small Animal Analysis Software, Hologic QDR-1000 System)

2.4. Statistical Analysis

The statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 27 software (IBM, Armonk, NY, USA). Normality of the data was analyzed using the Shapiro-Wilk test. One-way ANOVA and post hoc Tukey's test were used to analyze the significant differences in the normally distributed variables of interest between the study groups. General linear (repeated) measures were also used to analyze the variables of interest between the group. All data were presented as mean and standard error of mean (SEM). The differences were considered statistical at $p < 0.05$.

3. Results

The body weight of rats from Table 1 showed the highest weight in N group is comparable to CM, CS, HM and HM. The body weight of CM rats was significantly lower (17.31%) than the non-treated group CS and the results of the HM group have significantly 12.69% lower body weight compared to HF group ($p < 0.05$). HM rats fed with 10% roasted barnyard millet have a smaller abdominal circumference ($p < 0.05$) compared to non-supplemented groups HF at week 16. The systolic blood pressure of the rats was at highest in group HF ($p < 0.05$). From Table 1, there were comparison value of systolic blood pressure between the group given with 10% roasted barnyard millet where the HM group showed significantly lower blood pressure than the HF group ($p < 0.05$).

Table 1. Body weight, abdominal circumference, blood pressure, total fat mass and area under the curves blood glucose concentration of OGTT at week 16.

Variables	N	CM	CS	HM	HF
Body weight, g	468.07 ± .0.57 [a]	317.85 ± 2.05 [d]	384.41 ± 0.70 [c]	294.17 ± 1.06 [e]	336.94 ± 1.36 [b]
Abdominal circumference, cm	19.13 ± 0.30 [a]	14.81 ± 0.41 [bc]	16.75 ± 0.49 [bc]	14.63 ± 0.65 [c]	17.14 ± 0.94 [ab]
Systolic blood pressure, mmHg	103.29 ± 5.54 [b]	90.50 ± 4.00 [b]	102.86 ± 4.80 [b]	99.36 ± 5.16 [b]	160.32 ± 7.00 [a]
Total fat mass, g	61.73 ± 4.73 [ab]	42.96 ± 4.03 [b]	57.69 ± 6.12 [ab]	45.97 ± 8.20 [b]	82.06 ± 14.72 [a]
Area under the curve, mmol/Lxmin	567.64 ± 11.47 [a]	566.14 ± 17.7 [a]	587.36 ± 9.49 [a]	546.64 ± 11.16 [a]	561.43 ± 23.79 [a]

[1] Values are expressed as mean ± SEM, n = 7. Means with different superscripts between the row (a, b, c, or d) have significant differences at $p < 0.05$. N, normal pallet diet feed rats; CM, corn starch diet feed rats with 10% millet in food; CS corn starch diet fed rats; HM, high carbohydrate high fats diet with 10% whole grain barnyard millet; HF, high-carbohydrate, high-fat diet fed rats. All the values are taken at week 16 of experimental protocol. The area under the curve was for blood glucose concentration for oral glucose tolerance test.

The fat mass was the highest in group HF at week 16. The data in Table 1 shows that the total fat in HM was significantly lower compared to the HF group. Referring to Figure 1, the area under the curves of oral glucose tolerance test showed significant differences between week 8 and week 16 for group N and HM.

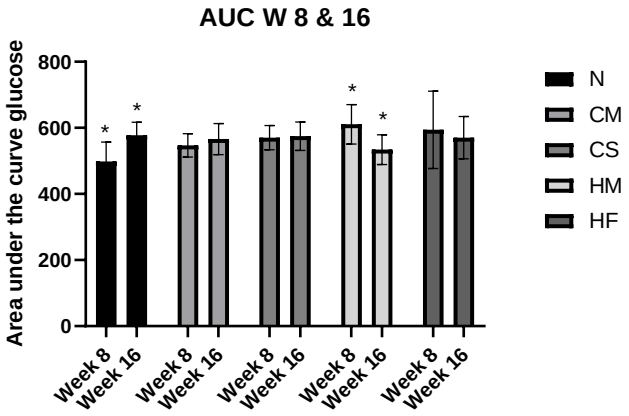

Figure 1. Area under the curves (AUC) for glucose tolerance test at week 8 and week 16 for each group. Data were presented as mean and standard error mean (SEM). The * symbols showed significant differences between week 8 and week 16 for each group ($p < 0.05$).

4. Discussion

A long-term effect of consuming an unhealthy diet leads to metabolic dysfunction. A suitable model that mimics all the symptoms of human metabolic syndrome is needed to test the potential pharmacological interventions in reversing the metabolic dysfunctionality. Feeding the rats with high content of simple sugar sourcing from sucrose and fructose, added with saturated and trans-fatty acids, would induce metabolic disorders such as abdominal obesity, high blood pressure, impaired glucose tolerance, and hyperlipidemia. Using this diet-induced metabolic syndrome rats' model, we can observe the responses of the additional of roasted whole-grain barnyard millet into the diet to the metabolic parameters of the rats. The main goal of this study was to evaluate the potential of roasted whole-grain barnyard millet in reversing the metabolic disorder.

This study used the corn starch diet as a control diet because of its slow digestible glycemic carbohydrate properties. These slowly digestible carbohydrate properties from the corn starch provide a control for a high carbohydrate high fat (HCHF) diet [3] where

the source of carbohydrates in HCHF diet are simple sugar fructose and sucrose from condensed milk. The carbohydrate content in corn starch does not induce metabolic syndrome compared to fructose [10]. This criterion makes the corn starch diet a suitable control diet for HCHF diet because the only differences between the diet are the carbohydrate and the usage of ghee as the high-fat source.

In the HCHF diet, high fats and high sugar exist in the diet provide more calories than the animal needs. The excess energy will be stored in adipocyte fat cells and lead to excessive adipocytes, which can cause obesity and induce metabolic disorders [11]. Our study showed that the HCHF diet feed rats (HF group) resulted a higher abdominal body circumference, blood pressure and fat mass (Table 1) compared to the control group of CS, which could indicate the results of excessive calories from the diet consumed by the animal. The results of abdominal circumference, blood pressure and the fats deposition for metabolic syndrome diet-induced rats shows the same results from the study conducted by Wong et al. (2017) [9].

The potential effect of barnyard millet was assessed from group HM and CM in this study. From Table 1, group given with roasted barnyard millet shows a lower body weight. Our results were similar to the previous study conducted using finger millet non-starch polysaccharide extract to the high-fat diet-induced rats [12]. However, there are contradictions with other findings using whole grain finger millet to the obesity-induced rats model [13]. The contradiction from previous findings could indicate that the cooking method of roasting could enhance the antioxidant properties [14] and derive higher phenolic compounds in the millet resulting from the breakdown of membranes and cellular constituents during heating compared to non-roasted treatment [15] which could possess the anti-obesity effect. The body weight reduction may also be due to the properties of bound phenolic compounds in the barnyard millet [16].

Rats fed with normal food pallets showed the highest weight in this study was similar to the previous findings [9] comparing the metabolic parameters of normal fed rats and HCHF diet-fed rats. It is postulated that the weight gain caused by the normal diet was due to the high protein and low-fat content in the normal diet, hence contributing to the development of higher muscle mass and low-fat mass of normal rats [9]. It is well known that the density of a fat-free body is higher compared to the fat-rich body [17]. Since the density is directly proportional to the mass, this explains why the normal fed rats resulted in the highest body weight.

The increase of abdominal circumference indicates the fat accumulation in the abdominal region of obese high carbohydrate, high-fat induced rats [18]. The previous study mentioned that the abdominal circumference of diet-induced metabolic disorder is directly proportional to the fat accumulation around the abdominal region [3]. Our study showed that the abdominal circumference of the treated group HM is lower than the non-treated group HF. Thus, this indicates that there was a lesser fats accumulation at the abdominal site. This result was proven by the lower value of fat mass between HM and HF group, which was identified by the DEXA scanning. The anti-hyperlipidemic properties of millet could be responsible for reducing the abdominal circumference and the fat mass of the rats. The inhibition of the hyperlipidemic effect of phenol content in the millet could promote the production of short-chain fatty acid by altering the good bacteria profile in gut [19]. Production of certain SCFA by the gut bacteria was well associated with improving health conditions, especially in the lipid profile context [20]. Besides, the high fiber content in the millet also contributed to the anti-hyperlipidemic properties [21]. The consumption of a high fiber content diet believed to offer a protective effect against lipid deposition [22].

The high blood pressure in HF group could possibly indicate the rat's developed hypertension caused by the high carbohydrate high fats diet. The elevation of high blood pressure due to HCHF diet show agreeable results from the previous study [9]. The lower value of systolic blood pressure of HM group than HF group shows a similar result from other findings using the spontaneously hypertensive rats model [23]. The antioxidant and

anti-inflammatory effect from the barnyard millet could be the key properties for these differences by improving the vascular condition of the metabolic syndrome rats [24]. The AUC of N group shows an increasing trend between week 8 and 16, possibly due to the development of metabolic disorder caused by the standard food pellet given. According to previous literature [25], standard rat food showed evidence in development of metabolic morbidity to the rats.

From Figure 1, the area under the curve in HM shows a significant reduction between week 8 and week 16. A previous study using the barnyard phenolic extract reported that the blood glucose level of drugs induced diabetic rats give a better blood glucose profile after a certain period [26] and the results obtained from the study are similar to our findings. It is suggested that the properties of *p-coumaric* and chlorogenic acids can be found majorly in the Barnyard millet responsible for inhibiting protein glycation, which is the key mechanism to prevent hyperglycemia abnormalities [27].

5. Conclusions

In conclusion, this study accentuates the importance of using animal models to investigate the potential properties of the roasted whole-grain Barnyard millet to the metabolic parameters of metabolic syndrome rats. We can conclude that the 10% roasted barnyard millet could potentially reduce the body weight, abdominal circumference, fat mass, and blood glucose level of diet-induced metabolic syndrome rats.

Author Contributions: S.R.S. for conceptualization and methodology; M.S.M performed the experiment and drafted the manuscript; S.R.S., N.H.S. and F.A. conceived the study and provided critical review to the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: We thank the Office of the Deputy Vice Chancellor (Research and Innovation) Universiti Putra Malaysia for the fundings required for this study.

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

Acknowledgments: We thank Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia for the help with blood pressure analysis.

Conflicts of Interest: The authors declare no conflict of interest.

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