Journal of Pharmaceutical Research International

24(5): 1-7, 2018; Article no.JPRI.45285
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Lipid-lowering Effects of Foxtail Millet (Setaria*italica*) and Quinoa (*Chenopodium quinoa*wild) in Pre-diabetics

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Authors' contributions

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

Article Information

DOI: 10.9734/JPRI/2018/45285
Editor(s):
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Complete Peer review History: http://www.sciencedomain.org/review-history/27913

Original Research Article

Received 12 October 2018
Accepted 14 December 2018
Published 21 December 2018

ABSTRACT

The present investigation was aimed to study the effect of foxtail millet (FM) and quinoa (QA) food supplementation on lipid profile in non-diabetic subjects with impaired glucose tolerance (IGT). A cross over design was used for this study. Ten subjects were given 65 g of QA for 30, days and with a wash of 15, FM was given for another 30 days and lipid levels were studied. Supplementation of QA followed by FM significantly(p=.05) reduced all lipid fractions. QA consumption significantly reduced all fractions of lipids than that of FM except HDL-C. FM

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consumption resulted in significant reductions in TC and LDL-C but not in other fractions. Though not significant, a higher increase in HDL-C was exhibited by FM (1.7 mg/dl) than by QA (0.5 mg/dl). These differences can be attributed to the higher dietary fibre and lower carbohydrate content of QA than that of FM. During the 60 days, consumption of QA and FM was found to have an overall benefit of lowering TC, TG, LDL-C, VLDL-C and HDL-C by 10.77, 6.83, 3.39, 14.63 and 7.71% respectively. The study demonstrated that consumption of QA and FM are beneficial for the risk reduction of higher lipids in the human subjects.

Keywords: Quinoa; foxtail millet; prediabetics; lipid profile.

1. INTRODUCTION

Prediabetes (intermediate hyperglycaemia) is a high-risk state for diabetes that is defined by glycaemic variables that are higher than normal, but lower than diabetes thresholds. 5–10% of people per year with prediabetes will progress to diabetes, with the same proportion converting back to normoglycemia. Prevalence of prediabetes is increasing worldwide, and experts have projected that more than 470 million people will have prediabetes by 2030. Prediabetes is associated with the simultaneous presence of insulin resistance and β-cell dysfunction—abnormalities that start before glucose changes are detectable. Higher levels of lipids were reported in prediabetics [1]. The prevalence of dyslipidemia ranged from 75.7% in urban Maharashtra to 87.2% in urban Chandigarh, and 76.5% in rural Tamil Nadu to 81.1% in rural Chandigarh. The prevalence of coronary artery disease was higher among diabetic subjects compared to normal with Maharashtra having the highest prevalence. For prediabetic individuals, lifestyle modification is the cornerstone of diabetes prevention, with evidence of a 40–70% relativerisk reduction. It is evident that lifestyle intervention can prevent or at least postpone type 2 diabetes [2]. There is always a huge interest exists to identify newer effective therapeutic agents that can be easily adopted and used regularly to control a progression of T2DM in the prediabetic population. Millets and pseudocereals provide an excellent source of nutrients and phytochemicals which are reported to have several health benefits.

Good amounts of therapeutic compounds are found both in foxtail millet and quinoa such as dietary fibre, phenolics, protein and amino acid and fatty acid profile etc. are identified and has been reviewed. There are numerous studies showing the cholesterol- and blood glucose-lowering effects in animal models but are scarce in human subjects [3]. There is growing interest among scientists to further understand the efficacy of these millets and pseudo cereals in the risk reduction of many dysregulations in the human body. To our knowledge, there are no studies comparing the efficacy of quinoa and foxtail millet consumption on lipid levels, hence the present study was conducted to study the effect of these grains on the lipid fraction.

2. METHODS

2.1 Nutrient Analysis

The proximate analysis of quinoa and foxtail millet was assessed by standard procedures for moisture [4], protein [5], fat, dietary fibre, total ash andcarbohydrates [6].

2.2 The Biochemical Parameters

Cholesterol [7,8] LDL [9], triglyceridesby glycerol phosphate oxidase-phenol amino antipyrine method by using enzymatic kit and HDL-cholesterol by cholesterol oxidase/ phenol aminoantipyrinemethod [10] were assessed by standard protocols.

2.3 Study Design

Crossoverthe study.

2.4 Inclusion and Exclusion Criteria

Inclusion:

Non -diabetic people with Impaired glucose tolerance (men and women)
Age 20-50 years
Non-smoking
Not taking medication
Non- dieting (weight stable in prior 3 months)
BMI 18-27
English literacy

Exclusion:

Do not regularly consume breakfast
Food allergies to ingredients found in the study products
BMI <18 or >27
Diagnosed cardiovascular disease, renal disease, hepatic disease, or diabetes mellitus.
Cancer in the previous 5 years.
Any gastrointestinal disease or condition.
Recent bacterial infection (< 3months).
History of drug or alcohol abuse in prior 6 months.
Use of lipid-lowering, anti-hypertensive, or anti-inflammatory steroid medication.
Eating disorder.
Women who are pregnant or lactating.
Women with irregular menstrual cycles.

2.5 Study Protocol

Blood sampling – normal diet (1-15th day)-(Base line period)
Blood sampling – Quinoa (16th -46th day)
Blood sampling – normal diet – Wash out period (47th – 62nd day)
Blood sampling- Foxtail millet (63rd – 93rd day)

2.6 Selection of Subjects

Subjects were recruited from among the attendants and family members of the diabetic patients regularly attending the outpatient facility at Hyderabad multi-speciality hospital under the supervision of the medical officers. Subjects with fasting glucose levels >100 mg/dl and ≤ 125 mg/dl and post prandial being ≥ 140 mg/dl and < 200 mg/dl are considered as prediabetes [11].

Prior to the study, subjects were given an information sheet containing details about the project regarding a total number of visits, the nature and the purpose of the procedures involved, the benefits, risks and the potential side effects associated with the grain used in the study. 12 non-diabetic subjects with impaired glucose tolerance (allowances of drop outs included) of males and females with age ranging from 25- 50 yrs were selected and informed consent was taken.

2.7 Supplementation

Before supplementation of the grains to the prediabetics, acceptability tests were conducted by sensory evaluation for cooked rice using a 9-point hedonic scale in a semi-trained population of prediabetics [12]. All the subjects were supplied with 65 g of quinoa (QA) and foxtail millet (FM) sachets along with the cooking procedure and asked to consume during lunch, in place of their regular rice, for 30 days. Initially, quinoa and later foxtail millet were consumed after a 15-day wash off period. During the study, the subjects were monitored by home visits and telephone. The biochemical parameters, FG, PG, lipid profile, C- reactive protein were studied at baseline (T1), after the quinoa supplementation for 30 days (T2), after the wash off period for 15 days (T3) and finally after the foxtail millet supplementation for 30 days (T4).

2.8 Statistical Analysis of the Data

The results were statistically analyzed using STAT GRAPHICS centurion version 17.1.11. One-way repeated measures ANOVA technique was used to find out the significant effect of Foxtail millet and Quinoa on lipid levels on Prediabetes people with impaired glucose tolerance. Post hoc test for multiple comparisons was conducted using Fishers LSD.

3. RESULTS AND DISCUSSION

The nutrient composition of quinoa and foxtail millet is presented in Table1.All the nutrients were higher in quinoa than in foxtail millet (P=.05), except carbohydrate. Though minerals were not analyzed, it can be predicted that quinoa might contain more minerals based on the ash quantity. The total protein content in quinoa is higher than that in barley (11.0% dm), rice (8.8% dm), corn (10.5% dm), rye (dm 11.6%), and sorghum (12.4% dm), getting close to wheat (14.8% dm) [13, 14, 15]. Deepali et al. [16] reported a mean protein of 11.33 and dietary fibre of 8.17 in six foxtail millets grown in India.

3.1 Total Cholesterol

The Total cholesterols (TC) was reduced from 202.40 to 181.80(10.8%) and 187.70 to 180.60(3.78 %) after QA and FM consumption respectively for 30 days (Table 2). This indicates a higher effect of QA than that of FM. Though the dietary fibre content in FM is lower than the barnyard millet, still it caused a greater reduction in TC than that of barnyard millet, indicating apart from dietary fibre there are other compounds like protein-lipid complex and soluble fibre might have contributed to this reduction. FM was found to have a better effect on the reduction of TC

compared to proso millet and sorghum in hyperlipidemia-inducedrats [17].

Table 1. Nutritional composition of quinoa and foxtail millet

Nutrients	Quinoa*	Foxtail millet *
Carbohydrates(g)	63.18±0.22	69.48±0.21
Protein(g)	17.18±0.34	14.03±0.32
Fat (g)	6.50±0.44	4.66±0.34
Moisture	11.00±0.54	10.82±0.22
Ash(g)	2.14±0.33	1.01±0.11
Dietary fibre (g)	15.80±0.31	10.94±0.10

**Significant at P=.05*

3.2 HDL- cholesterol (HDL-C)

The mean HDL- C of baseline was 36.30 mg/dL which was increased gradually to 36.80 mg/dL, 37.40 mg/dL and 39.10 mg/dL (7.71%) by the end of the study (Table 2). Though not significant, FM caused a higher (4.55%) increase in HDL-C than that of quinoa (1.38%). A non-significant increase of 2.3% HDL-C was reported after consumption QA bars by normal population for 30 days [18]. No significant effect was found in overweight and obese subjects with 50 g of QA supplementation for 12 weeks on HDL-C [19]. From the present study, it was clearly observed that with respect to HDL-C improvement FM is beneficial than that of QA. However, in order to establish a significant effect, a long-term trial is warranted.

3.3 LDL- cholesterol (LDL- C)

The effect was different in other lipid fractions, where a higher effect was observed with QA than that of FM. A 8.0 mg reduction (5.76%) occurred due to QA against a reduction of 3.5 mg/dl (1.54%) due to FM consumption, indicating a significantly higher effect of QA on LDL-C reduction (Table 2). QA consumption effect was higher than the net effect of both QA and FM consumption (3.39%), indicating a regular consumption of QA is more beneficial for the risk reduction of LDL-C. Many prospective studies have shown that high serum concentrations of LDL-C are a major risk factor for coronary heart disease (CHD). Vanessa et al. [20] reported a significant reduction in total cholesterol (P=0.0008), triglycerides (P=0.001) and LDL-C (P=0.008 in coronary heart disease patients with quinoa diet for 120 to 200 days. Finger millet buns caused a reduction of 11.22% in diabetics [21]. This reduction may further sustain if the same diet continues which, reduces the risk of coronary heart diseases. All Adult Treatment Panels of the National Cholesterol Education Program reports have identified low-density lipoprotein cholesterol (LDL-C) as the primary target of cholesterol-lowering therapy. Many prospective studies have shown that high serum concentrations of LDL-C are a major risk factor for coronary heart disease (CHD). A large number of RCTs, moreover, have documented that lowering of LDL-C levels will reduce the risk for major coronary events [22].

3.4 VLDL - cholesterol (VLCL-C)

There is no significant effect FM consumption on VLDL-C, only a marginal reduction with 0.8 mg/dL was observed. From T1 to T2 there was a reduction of 4.1 mg/d. Though FM could not improve the VLDL-C it has inhibited the increase of VLDL-C (Table 2).

3.5 Triglycerides (TG)

Significant effect of QA and FM consumption was also observed on TG by 6.1 and 4.16% reductions respectively. The reduction of TG from T1 was reversed by 5.0 mg/dL indicating the QA with drawl effect, however again reduced soon after FM consumption, indicating a very strong effect of FM on the reduction of TG. Overall there was a net reduction of 10.3(6.83%) due to QA and FM for 60 days (Table 2)

3.6 Lipoprotein Ratios

The total/high-density lipoprotein (HDL) cholesterol ratio, known as the atherogenic or Castelli index and the LDL/HDL cholesterol ratio are two important components and indicators of vascular risk, the predictive value of which is greater than the isolated parameters. In this respect, an increase in total cholesterol concentration, and specifically LDL cholesterol is an atherogenic lipid marker, whereas reduced HDL cholesterol concentration is correlated with numerous risk factors, including the components of the metabolic syndrome, and probably involves independent risk. Non-HDL cholesterol, which is total cholesterol minus HDL cholesterol, is a measure of the cholesterol in LDL, IDL and VLDL particles. Non-HDL cholesterol has therefore been recommended as a secondary therapeutic target in individuals with high triglyceride concentration, and it has been suggested that it could be a surrogate marker of serum apoB concentration in clinical practice [23].

Table 2. Effect of QA and FM consumption on lipid fractions in prediabetic subjects

Parameter	T1	T2	T3	T4
TC	202.40±9.78 [b]	181.80±12.27[a]	187.70±9.85 [a]	180.60±9.24[a]
TG	150.70±4.87[c]	141.50±4.17[a]	146.50±4.12[b]	140.40±4.59[a]
LDL-C	138.80±4.15[b]	130.80±4.11[a]	136.20±3.79 [b]	134.10±3.97 [a]
HDL-C	36.30±1.72[a]	36.80±1.05[ab]	37.40±1.58[ab]	39.10±1.59 [b]
VLDL-C	36.80±2.55[b]	32.70±2.21 [a]	32.80±2.16[a]	32.00±2.40[a]
Non-HDL-C	166.10±9.99[a]	145±12.31[bc]	150.30±9.87[c]	141.50±9.24[bc]

T1: base line, T2: After quinoa consumption, T3: Foxtail millet consumption, T4: After foxtail millet consumption
**The similar superscripts in the same row are not significantly different from each other*

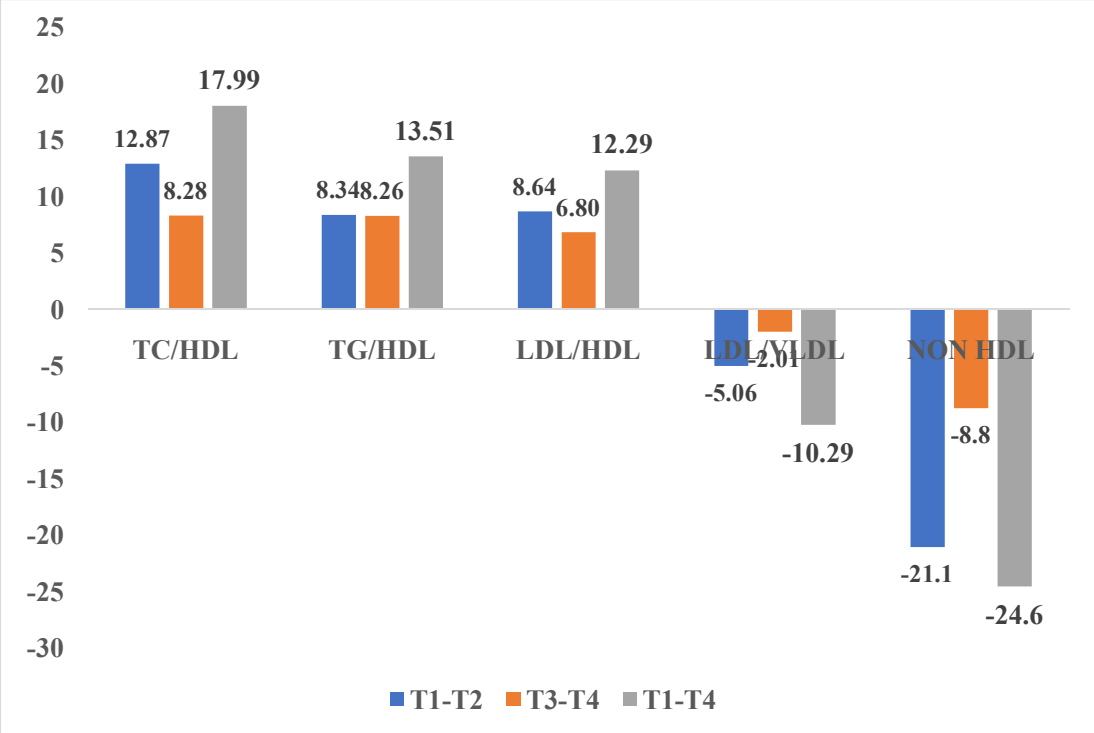

Fig. 1. Percent changes in the lipid fraction ratios during study period
T1: BASE LINE, T2: AFTER QUINOA CONSUMPTION, T3: FOXTAIL MILLET CONSUMPTION, T4: AFTER FOXTAIL MILLET CONSUMPTION

From the lipoprotein values of the current study, we calculated different ratios for better understanding of the effectiveness of the QA and FM consumption and presented graphically in Fig 1. The TC: HDL-C ratio decreased from 5.67 to 4.94 and from 5.07 to 4.65 due to QA and FM consumption respectively as shown in table 4.8 and by the end of 60 days consumption of both grains could reduce 1.01 units. TG: HDL-C and LDL-C: HDL-C ratios reductions briefly; 0.35, 0.33, 0.57 and 0.34, 0.25,0.48 after QA, FM and QA& FM consumption respectively. The LDL-C/HDL-C ratio reflects the two-way traffic of cholesterol entering and leaving the arterial intima in a way that the individual levels of LDL-C and HDL-C do not [24]. The ratio was especially accurate at predicting risk among those who also had elevated triglyceride levels. It was found that by using the LDL-C/HDL-C ratio along with fasting triglyceride concentration [18]. The LDL-C: VLDL- C ratio increased by 0.2, 0.09 and 0.41 units after QA, FM and QA& FM consumption respectively. Non-HDL-C reduced by 21.1, 8.8 and 24.6 units after QA, FM and QA& FM consumption respectively (Fig. 1).

From the present study, it was observed that during the wash off period the TG, LDL-C and TC, VLDL-C levels were reduced significantly and non-significantly respectively and an

increase in HDL-C was observed. Among the lipid fractions efficacy of QA was in the order of; VLDL-C>TC>TG>LDL-C. The TC: HDL-C ratio decreased from 5.67 to 4.94 and from 5.07 to 4.65 due to QA and FM consumption respectively and 1.01 units by the end of 60 days consumption of both grains. The changes in other ratios due to QA and FM consumption be can be observed from Table 2.

4. CONCLUSION

Both quinoa and foxtail millet grains are gaining popularity among the consumers and scientific community due to its potential nutrient and bioactive composition. The present study demonstrated that the consumption of quinoa and foxtail millet exerted positive benefits on lipid fractions in prediabetics. Though higher benefits were observed with quinoa over foxtail millets except for HDL-C, it is assumed that a regular consumption of these grains can contribute to the risk reduction of prediabetics turning into diabetics. A long duration study might confirm this impact. As the grain is used in the form of rice, it is easier to adapt though substitution or replacement to regular polished rice.

ETHICAL APPROVAL AND CONSENT

The study was approved by the by the advisory committee specifically formed for the study (endorsement No. B-133/PG/A2/2-15 dated 14-10-2016.An additional approval was taken from the multi-speciality hospital, Hyderabad Informed consent was obtained from the subjects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27913