



Effect of Amaranth Grain Consumption on Lipid Profile of Hypercholesterolemic Subjects

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Abstract

Introduction: An increase in Obesity and increased risk for chronic diseases has sparked global interest in less consumed traditional foods such as Quinoa and Amaranth. Amaranth grain is a pseudo-cereal with high quality gluten-free protein. It is consumed as a cereal, popped or in the form of a sweet preparation for religious fasting occasions in India. Animal studies suggest that amaranth seed consumption improves blood glucose and lipid profile. The present study was carried out in India with the objective of assessing the impact of amaranth grain consumption on fasting lipid profile and glucose concentration of hypercholesterolemic subjects.

Materials and Methods: Nineteen subjects aged between 40-65yrs with hypercholesterolemia, fasting plasma cholesterol \geq 200mg/dl were recruited. Informed consent was taken from the willing subjects, and popped amaranth grain 60g/day were provided for a period of eight weeks to replace regularly-consumed cereals in their diet.

Before and after 8 weeks of amaranth grain consumption anthropometric parameters, fasting blood glucose and body composition by bio impedance method were measured.

Results: No significant changes in weight and body composition were observed. However, plasma total and LDL-cholesterol and fasting plasma glucose concentrations showed significant increases after 8 weeks of amaranth grain consumption.

Conclusion: Consumption of popped amaranth grain for 8 weeks in humans did not decrease blood lipid levels, however a small increase in female subjects was observed. The significance of this observation is not known.

Keywords: Amaranth Seeds; Fasting Plasma Glucose; Total Cholesterol; HDL-Cholesterol

Introduction

The global increase in obesity and chronic diseases, has led to an increased awareness and consumption of less common traditional foods with potential health benefits. In the Indian context amaranth grain, a promising pseudo-cereal with good quality protein and potential to increase bile excretion and decrease LDL-cholesterol in animals, can be considered as one such food [1-3]. The common species grown in India are *Amaranthus cruentus*, *Amaranthus gangeticus* and *Amaranthus spinosus*. Both leaves and grain are used in Indian cuisine in southern and western parts of India.

Quinoa and amaranth have nutrient composition similar to cereals and 100g of amaranth grain has 13.3g protein, calcium (162mg) and Iron (8.02mg) thereby making it a rich source of protein and minerals. Further the amaranth protein is good quality protein as it is rich in lysine 5.5g/100g of protein [4]. While amaranth leaves are extensively used all over India as stir-fried vegetable, cooked with dal (pulses), amaranth grain, locally known as rajgira seeds is used in western India, during religious fasting and in the monsoon months. Popped amaranth grain-laddus are (sweet preparation), usually consumed only in western and central parts of India.

Animal studies by Kim et al. have shown that feeding of both amaranth grain and its oil, to the diabetic rats, resulted

in the improvement in hyperglycaemia and reduced plasma cholesterol levels [5,6]. Berger et al. have demonstrated in hamsters the cholesterol lowering effects of amaranth seeds and oil by influencing the absorption of cholesterol and bile acids, cholesterol distribution in lipoprotein, hepatic cholesterol content and cholesterol biosynthesis [7,8]. In contrast, de Castro has not found any cholesterol lowering effects of amaranth oil, despite increased excretion of bile acids, when hamsters are fed on high saturated fat and cholesterol diet [9]. The reported beneficial effects of this nutrient-rich pseudo-cereal have been more widely studied in animals, and very limited data are available on the health effects of amaranth grain consumption in humans. The present study was planned to assess the impact of amaranth grain intake on lipid profile and fasting plasma glucose levels in free living hypercholesterolemic subjects.

Materials and Methods

Sensory Evaluation of Amaranth Recipes: Recipes were prepared in the institute's metabolic kitchen with amaranth grain and were tested for sensory characteristics and acceptability. Popped amaranth seeds and roti (unleavened flat bread) were given to 30 subjects. Based on individual acceptability, popped seeds or powdered popped seeds were provided to the subjects for consumption in the form of roti or popped seeds as such.

On a pre-heated metal pan, a batch of 50-60g of amaranth grain were taken and continuously stirred for 1-2 minutes (approximate time required for popping of all the seeds) and cooling seeds to the room temperature, then seeds were powdered by grinding in a mixer. Majority (65%) of the subjects preferred consumption of popped seeds as such, whereas 35% subjects consumed as roti prepared with flour. Both forms were well accepted by the participants, and they willingly consumed popped amaranth seeds/powder continuously for eight weeks. Dietary fibre both soluble and insoluble content of amaranth grain and popped seeds was estimated using rapid enzymatic assay method [10].

Each subject was given 60g of flour/popped grain pack per day for a period of eight weeks. Investigator ensured the compliance at the time of contact, by enquiring about the regularity of the consumption. Details regarding the other regular food consumption pattern pertaining to oil, fruits, vegetables and information about physical activity of all the nineteen subjects were collected to assess any changes in the food intake and exercise, during the study period.

Subjects

Apparently healthy volunteers (nine males and ten females), with fasting plasma total cholesterol >200mg/dl, aged between 40-65 yrs., and not on any lipid or cholesterol lowering drugs were enrolled. None of them had diabetes or thyroid disorders. The 19 subjects were informed about the purpose of the study and informed consent was obtained through duly signed consent

forms. The study was cleared by the institute's ethical committee for humans of the national institute of nutrition, Hyderabad, India (No.3/2011) and conducted according to the guidelines of Helsinki declaration [11]. The volunteers were asked to consume one packet (60g of flour)/day replacing regularly- consumed cereal for breakfast/ lunch/ dinner along with other preparations for a period of eight weeks. Ensured the total consumption by collecting the feedback information on left over grain at end of two-week period. We observed that all the subjects consumed about 95 to 100% of the grain provided and relished its consumption in the form of rotis in the dinner or along with milk as a breakfast item. As the subjects are in free living conditions they were advised to continue their routine diet and physical activity.

Body weight and body composition were recorded, by bio-impedance method by using Tanita BC-418, a dual frequency segmental body composition analyser. Blood samples were collected before initiation and after eight weeks of amaranth cereal consumption. Blood samples were collected after 12h fasting for the determination of plasma lipids. Commercial enzymatic kit methods were used for total and HDL-C and triglyceride estimations. The LDL-C levels were determined through the Fried Ewald, Fredrick, and Levy (1972) equation for TG lower than 400.0 mg/dL: $[LDL-C = total -C - (HDL-C + triglyceride/5)]$ [12]. The content of very low-density cholesterol (VLDL-C) was evaluated by the formulae: $VLDL-C = TG/5$ for TG lower than 400.0 mg/dL. Data are expressed as mean \pm Standard Deviation (SD). Glucose was estimated by glucometer and dietary fibre was analysed by enzymatic method (10) in both raw and popped grain.

Sample size

Sample size was calculated by taking into consideration the possible dropout rate of 10% and to detect the difference of 26 mg/dL (the change or difference between before and after supplementation) in plasma total cholesterol and standard deviation of 35 for total plasma cholesterol and 95% CI and 80% power, 14.2 subjects have to be recruited into the study 30% attrition 18.2 subjects were required, 19 subjects were recruited into the study.

Statistical analysis

The statistical analyses were performed using the SPSS 21 software. The comparison of initial and final values was done using paired 't' test. The level of significance was set at $p < 0.05$.

Results

The nutrient content of the 60g of seeds provided per day is given in table-1 [4]. Sixty grams amaranth grain provided 213.5 K.Cal energy and 8g of protein, 3.3 g of fat, and 36.9g of carbohydrate and 4.5g of dietary fibre [4]. The dietary fibre was analysed in raw and popped amaranth grain, which was almost, double than the reported value (table-2). Based on the dietary fibre content of sixty grams' grain consumed by the subjects, dietary fibre content was approximately 9-10g.

Amaranth raw grain	Protein (g)	Fat (g)	CHO (g)	Energy (Kcals)	Total Dietary fibre (g)	Soluble Dietary fibre (g)
60g	7.96	3.33	36.88	213.50	4.48	1.00
Reference :Indian food composition tables (2017)						

Table 1: Macro nutrient composition of the sixty grams of amaranth grain powder provided for daily consumption.

Amaranth Grains	Insoluble Dietary Fibre (g)	Soluble Dietary Fibre (g)	Total Dietary Fibre (g)
Raw grain (60g)	8.814	0.942	9.756
Popped grain (60g)	9.822	1.650	11.472

Table 2: Dietary fibre content in amaranth seeds.

Subjects consumed 95-100% of the food provided to them. Details regarding other food consumption revealed that majority of the subjects consumed mango, a seasonal fruit during the study period. Other than regular mango fruit consumption, other foods consumed by them remained the same, during the study period. Initial and final anthropometric and body composition parameters are given the table 3,4. There were no significant differences in weight, fat percent, and trunkal fat, during the study period in the subjects.

Parameter	Initial values		Final values		Initial vs final P value	
	Male (9)	Female (10)	Male	Female	Male	Female
Age (yrs)	52.7±7.25	53.7±5.96				
Height (cm)	164.3±4.92	157.7±7.00				
Weight (kg)	74.6±14.76	70.4±10.87	75.2±14.33	70.2±10.53	0.123	0.774
BMI (kg/m ²)	26.3±3.04	28.3±3.79	26.3±2.48	28.2±3.59	0.779	0.311
Fat (%)	26.6±4.66	40.9±3.00	27.0±3.00	41.1±3.37	0.612	0.370
Fat (kg)	18.9±4.75	29.0±6.02	19.3±3.34	29.2±6.33	0.538	0.206
FFM/LBM (kg)	51.7±5.36	41.4±5.13	51.9±5.35	41.3±4.83	0.700	0.492
Trunk fat (%)	30.1±5.53	39.9±3.56	31.0±4.29	40.0±4.08	0.401	0.735
Trunk fat (kg)	12.1±2.88	15.2±3.21	12.6±2.17	15.4±3.56	0.319	0.316
Number in the parentheses indicates the number of subjects. Values are mean ± SD						

Table 3: Effect of amaranth grain consumption on anthropometry and body composition by gender.

Parameter	Initial (19) (Mean±SD)	Final (19) (Mean±SD)	P value
Total- cholesterol ((mg/dl)	209.4±32.75	223.4±38.76*	0.005
HDL-cholesterol ((mg/dl)	38.4±12.07	37.7±10.82	0.658
Triglycerides (mg/dl)	100.0±56.51	111.5±58.51	0.080
Fasting glucose (mg/dl)	94.6±43.56	105.1±39.74*	0.023
LDL-cholesterol(mg/dl)	151.0±32.19	163.4±35.21*	0.039
VLDL-cholesterol(mg/dl)	20.0±11.30	22.3±11.70	0.080
Number in the parentheses indicates total number of subjects			

Table 4: Effect of amaranth grain consumption on lipid parameters of male and female together

Initial and final fasting plasma lipid and glucose concentration of all the subjects (male and female together) are given in Table-4. Plasma total cholesterol and LDL-cholesterol and glucose concentrations showed significant increases, after the consumption of amaranth grain for eight weeks. The plasma total cholesterol showed a significant increase in only female subjects but not the male (data not shown).

Discussion

In the present study among 19 human subjects the effect of consumption of 60g of cereal showed a small but significant increase in total and LDL cholesterol. The study of Cinthia Baú Betim Cazarin et al., has observed that feeding of extruded amaranth grain as a snack to normo-cholesteremic rats has significantly increased the faecal excretion of bile acids and decreased plasma LDL-cholesterol, without impacting plasma glucose concentration and liver lipid content [13]. On the contrary, the results of our study in hypercholesteremic subjects indicated that chronic (eight weeks) consumption of amaranth grain significantly increased fasting plasma total and LDL-cholesterol and glucose concentration. Capriles et al. (2008) have reported that when Amaranth grain is processed it increases digestibility and the glycemic index of both popped and roasted amaranth by in vitro method. This probably explains the observed increase in blood glucose levels of the subjects in the present study.

Kim et al. (2006), have observed improved lipid and glucose levels in diabetic rats [14]. The beneficial effects have been attributed to the antioxidant and polyphenolic compounds (Muyonga, J. H., Andabati, B., & Ssepunya, G. 2014) [15].

Our results are in contrast to those of Chávez-Jáuregui et al., who have reported no changes in plasma lipid parameters, except decrease in plasma HDL-cholesterol of eleven hypercholesteremic subjects aged 30-65 yrs, who consumed 50g of defatted amaranth grain extruded snacks for sixty days [16]. The observed differences between these two studies could be due to variations in the duration of the study and the form in which amaranth grain are provided. In our study, popped amaranth grain/powder was given for consumption for eight weeks' period as against defatted amaranth grain extruded snack is given to hypercholesteremic subjects for sixty days in the other study [16]. Martirosyan et al. have supplemented hypercholesteremic subjects (2 males and 38 female) with amaranth seed oil at 3, 6, 12, 18g per day for a period of three weeks along with hypo-sodium and anti-atherogenic diet and life style modification and observed decreased plasma total cholesterol and LDL-cholesterol and blood pressure [17]. However, the results of our study cannot be compared with the above-mentioned study, as the study design and forms of supplementation are different. Further, the subjects were not given any suggestion to modify their dietary habits and life style patterns. Mithila and Khanum have reported decreased body weight, food consumption, plasma total

and HDL-cholesterol, triglycerides and glucose concentration in rats and based on these observations amaranth grain consumption has been recommended for weight reduction [18]. However, we have not found any such effects in the free living moderately-hypercholesteremic subjects, who consumed 60g popped amaranth grain/powder for eight weeks on the contrary, we observed small but significant increase in total and LDL-C. To understand the basis of the increase, gender-wise analysis for the plasma lipid parameters was performed (results not given) and we observed that female subjects showed significant increase in fasting plasma total cholesterol which persisted even after pooled analysis. The higher fasting blood glucose concentration in our study maybe due to the high glycemic index (97) reported by Chaturvedi et al. [19], for popped amaranth grain consumption with milk. Though this is the first study from India, where amaranth leaves and grain are consumed in routine diets of the population, the study design has inherent limitations, as food frequency data may not give us a true picture of the small changes in food intakes (as it is based on dietary recall) and control group is not included only pre and post differences were studied. Replacing the regularly-consumed cereal component of the diet with amaranth grain for eight weeks in the diet of moderately hypercholesterolemic subjects had no impact on body weight, body fat content and its distribution. However, significant changes in plasma lipid parameters, as evidenced by increased plasma total and LDL-cholesterol were observed.

Conclusions

In conclusion, the consumption of 60 g of amaranth grain daily (8 g of protein/day) for eight weeks did not significantly reduce total or LDL-C in moderately-hypercholesterolemia subjects. On the contrary, increase in total cholesterol, LDL-cholesterol and fasting glucose levels were observed. A significant increase in total cholesterol was observed in female subjects alone, which persisted even after pooled analysis. Even though, amaranth grain consumption has resulted in significant decreases in total and LDL-cholesterol in animal studies, similar effects were not seen in humans, who replaced cereals with 60g of amaranth grain per day. The increased digestibility coupled with increase in glycemic index could be a critical factor in the observed changes. However, the effects on plasma lipid levels are a paradox to the animal studies. Further studies with larger number of subjects and higher intake of amaranth grain are necessary to test the effects of amaranth on lipid metabolism in humans before considering its suitability as a replacement-cereal in the Indian diet.

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