### RESEARCH

Finger millet porridges subjected to different processing conditions showed low glycemic index and variable efficacy on plasma antioxidant capacity of healthy adults

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#### Abstract

Finger millet porridges (FMP), rich in nutrient and non-nutrient compounds have been used in the traditional food cultures in Asia. The aims of the study were to determine the effect of different processing conditions of finger millet grains on glycemic response, phenolic content and the antioxidant activities of FMP and to determine the short term and long term efficacy of its consumption on plasma antioxidant levels of healthy adults. Twelve types of FMP were prepared combining different processing conditions. Phenolic content of porridges as well as antioxidant activities were determined. The glycemic index (GI) and glycemic load (GL) values of FMP were also evaluated. The long term efficacy of FMP consumption on plasma glucose (PG), total cholesterol (TC) levels and plasma antioxidant capacity (PAC) of 18 subjects were investigated using a 24 weeks randomized cross-over study. The short term efficacy of porridge consumption on AC was determined. PAC was measured by trolox equivalent antioxidant capacity (TEAC) and ferric ion reducing antioxidant power (FRAP). All FMP exhibited low GI values (< 55) except the raw roasted flour which showed high and medium GI values for both particle sizes used. Parboiling of finger millet grains with 15 min steaming produced FMP with low glycemic response and possessed high PAC. Compared to baseline, PAC measured using FRAP and TEAC assays increased after 8 weeks consumption of porridge though significant changes were not observed for PG and TC levels. Furthermore, PAC was increased by 23 and 14% after 2 h of porridge consumption as measured by TEAC and FRAP, respectively. FMP consumption increased the plasma total antioxidant capacity of healthy adults. Further research on examining the potential of FMP on improving the antioxidant capacity in patients with diabetes is warranted.

Keywords: FRAP, Long term and short term efficacy, Randomized cross-over study, TEAC, TPC

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#### Introduction

Oxidative stress is known to contribute to the development of several chronic ailments such as obesity, hypertension, diabetes, certain types of cancers, cardiovascular diseases, arthritis, and neurodegenerative disorders associated with unhealthy diet and sedentary lifestyle. Thus, external sources of antioxidants are required to prevent oxidative damage in human body once natural antioxidant defense systems are challenged. Epidemiological evidences have shown that consumption of whole grains with fruits, vegetables, and legumes is protective against the occurrence of a multitude of disease states.

The recent knowledge of millets as a rich source of antioxidant phenolic compounds and essential nutrients have attracted the interest of consumers to its potential health benefits in preventing and managing a myriad of chronic disease conditions (Shahidi and Chandrasekara 2013; Devi et al. 2014; Bora et al. 2019). Furthermore, millet grain phenolics act as potential antioxidant compounds in food and biological systems (Chandrasekara and Shahidi 2012; Kumari et al. 2019a). Globally, finger millet (Eleucine coracana) ranks fourth in importance among coarse grains, after sorghum, pearl millets and foxtail millets and is grown by subsistent farmers in the semi-arid tropics in South Asia and Africa (Gupta et al. 2012). Due to their small seed size, finger millets grains are processed as whole grains. Finger millet, locally known as Kurakkan, is the most popular type among other underutilized cereals in Sri Lanka and has been a part of a variety of cuisines in traditional food culture.

Jayawardana et al. (2019) recently showed that three common finger millets varieties, *Ravi, Ravana* and *Oshadha* grown in Sri Lanka are good sources of dietary fiber, resistant starch, and minerals especially potassium, calcium, phosphorus and iron. In addition to nutrients finger millets contain non-nutrient bioactive compounds such as phenolic acids which are found in the soluble and bound forms, and flavonoids (Chandrasekara and Shahidi 2011; Shobana et al. 2013; Chandrasekara 2019). It has been reported that 71% of phenolic acids in finger millets are present in the soluble form and protocatechuic acid is the major hydroxybenzoic acid present. The major hydroxycinnamic acid reported in the bound form of phenolics was *trans* ferulic acid (Chandrasekara and Shahidi 2011).

Finger millet flour is used for different food preparations, namely flat unfermented breads (*Roti*), porridges (thick and thin), *Pittu, Dosai, Itly*, and several other sweetened snacks in Asia (Shobana et al. 2013; Kumari et al. 2019b). Finger millet porridges (FMP) are popular weaning food for infants and a nutritious food for pregnant mothers and elderly (Shobana et al. 2013). FMP is a nutritionally balanced meal and preparation of porridge is convenient compared to other dishes which use finger millets flour (Shobana et al. 2013). Furthermore, a recent study reported that thin and

thick FMP had more absorbable phenolic compounds which might contribute to high postprandial antioxidant activities compared to other food preparations using finger millet flour (Kumari et al. 2019b).

Previous studies have reported that starches present in finger millet based foods are less digested by amylase enzymes in vitro (Singhe and Ali 2006). The presence of undigestible starch or resistant starch is important in reducing the postprandial glycemic response. Foods prepared using varying processing conditions such as grinding of millet grains and roasting of flour showed different glycemic responses (Roopa and Premavalli 2008). Parboiling is a popular hydrothermal tratment used for paddy to improve its milling yield and nutritional quality. However, limited published information is available for finger millet parboiling (Dharmaraj et al. 2011; Bora et al. 2018). The use of different processing methods may help improve the quality of FMP for rendering health benifits.

Previous studies have shown the differences in glycemic response with different processing conditions and preparation methods of finger millet foods (Shobana et al. 2010; Jayasinghe et al. 2013; Pradeep and Yadahally 2015; McSweeney et al. 2017). In addition limited studies have reported evidences on glucose and cholesterol lowering effects, and antioxidant properties provided by finger millet based feeds (Shobana et al. 2010; Shahidi and Chandrasekara 2014). Therefore, the objectives of the present study were a) to determine the effect of parboiling of finger millet grains, roasting of finger millet flour with different particle sizes on glycemic response, phenolic content and the antioxidant activities of finger millet porridges, (b) to determine the short term efficacy of FMP consumption on plasma antioxidant levels of healthy adults, and (c) to determine the long term efficacy of regular consumption of FMP for 8 weeks on plasma antioxidant levels, blood glucose and total cholesterol levels of healthy adults.

#### Materials

#### Finger millet samples

Finger millet (*Eleucine coracana*), variety *Oshada* was procured from Seed Certification Regional Office of Department of Agriculture, Palwehera, Sri Lanka.

#### Chemicals

Folin-Ciocalteu's reagent and potassium hydroxide were purchased from Research Lab Fine Chem Industries, Mumbai, India. The compounds 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2' azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-azobis (2-methylpropionamidine) dihydrochloride (AAPH), trolox, ferric chloride, ferrous sulfate, hydrochloric acid, acetate buffer, amyloglucosidase,  $\alpha$ -amylase, methanol, ethanol and acetone were purchased from Sigma-Aldrich Co.3050 (St Louis, MO, USA). Glucose GOD-PAP ready to use liquid was procured from BIOLABO, (Maizy, France). Stanbio Cholesterol Liqui Colour Liquid was purchased from Stanbio Laboratory (Boerne, TX, USA. Prodigy no-coding blood glucose test stripes were purchased from Prodigy Diabetes Care, LLC (Charlotte, NC, USA).

#### Methods

#### Sample preparation

Finger millets cleaned to remove debris and soil particles were subjected on different processing conditions. Grains were dehulled using a rice polishing machine (Rice husker and polisher PM 500, Satake Engineering Co Ltd., Osaka, Japan).

#### Parboiling

Whole grains were soaked in distilled water for 12 h and steaming was carried out for two time periods, 15 and 30 min. Grains were sun dried to reach 12% moisture content and stored in air-tight containers.

#### Grinding

Raw and parboiled finger millet grains were ground (Phillips HR 2011, Koninklijke Phillips Electronics N.V.) to obtain two flour samples that passed through a sieve opening of 0.038 mm (small particle size) and 0.18 mm (large particle size) using two sieve sets (As 200, Retsch, Germany).

#### Roasting

Finger millet flour obtained by grinding of raw (non-parboiled) and parboiled grains were roasted on a metal pan for 5 min with continuous mixing.

#### **FMP** preparation

Twelve types of FMP were prepared including combinations of processing methods of finger millet flour namely flour made from parboiled and raw grains, roasted and unroasted flour, and flour with two different particle sizes. These different FMP were identified by FMP1 to FMP12 (Table 2). Finger millet flour (42 g) and coconut milk (7.5 g of commercial real milk powder in 50 mL of water) were used as raw ingredients for each porridge preparation. Finger millet flour and coconut milk were mixed with boiling water (50 mL) and mixture was boiled for 20 min with continuous stirring. All FMP were used to determine the glycemic index (GI) and glycemic load (GL), phenolic content and antioxidant activities. Previous studies showed that more than 75% of phenolic content of finger millet was in the soluble fraction (Chandrasekara and Shahidi 2010, 2011; Kumari et al. 2017, 2019b). Therefore, soluble phenolic compounds of FMP were extracted and used for determination of antioxidant activities.

#### Extraction of soluble phenolic compounds

FMP samples were transferred to -80 °C freezer after cooling to room temperature. All FMP samples were freeze dried at -55 °C, and 0.012 mbar (Alpha 1–4 LD plus CHRIST, Osterode am Harz, Germany) and defatted by blending with hexane (1:5 w/v, 2 min, two times) at ambient temperature (30 °C). Defatted FMP samples were packed in polythene pouches and stored at -80 °C until used within 1 week for extraction of phenolic compounds.

Defatted FMP sample (5 g) was mixed with 100 mL of 70% (v/v) acetone in a conical flask and placed in a shaking water bath (BT 680D, YIHDER Co. Ltd., Taipei, Taiwan) at 50 °C stirring at 175 rpm speed for 40 min. The resultant slurry was centrifuged for 5 min at 3000 g (Refrigerated centrifuge 3-18R TOMOS Life Science Group, Belmont, MA, USA) and supernatant was collected. The extraction procedure was repeated for two times. Combined supernatants were evaporated in rotary evaporator (IKA RV-10, IKA°-Werke GmbH & Co. KG, Staufen im Breisgau, Germany) at 40 °C at 125 rpm. Concentrated samples were freeze dried at -55 °C. Lyophilized crude phenolic extracts were stored at -80 °C until used for further analysis. During all stages extracts were protected from light by covering with an aluminum foil.

#### Determination of total phenolic content (TPC)

The TPC of each extract was determined using Folin-Ciocalteu's reagent using soluble phenolic extracts (2 mg/mL) as explained elsewhere (Kumari et al. 2017). The TPC were expressed using a standard curve prepared for ferulic acid as  $\mu$ mol ferulic acid equivalents (FAE) / g of dry matter (dm).

Determination of DPPH radical scavenging activity (DRSA) DRSA of phenolic extracts (1 mg / mL in methanol) was determined as explained by Kumari et al. (2017) The DRSA was expressed as  $\mu$ mol trolox equivalents (TE) / g (dm).

#### Determination of resistant starch content

Resistant starch content of each representative porridge sample was analyzed according to the AOAC International (2005) official method 2002.02. Freeze dried powder of porridge sample (1.0 g) was mixed with 4.0 mL of  $\alpha$  amylase (10 mg/mL) containing amyloglucosidase 3 units / mL in a screw cap tube followed by mixing using a vortex mixer. The mixture was incubated at 37 °C with continuous shaking at 200 rpm for 16 h and at the end, 4.0 mL of ethanol (95%) were added and the contents vortexed thoroughly. Resultant slurries were centrifuged at 1500 g for 10 min and the supernatants carefully decanted by inverting tubes on a paper towel. Then 2 mL of 2 M KOH and a magnet were added to each tube and dissolved the pellet followed by stirring for 20 min in an ice bath using a magnetic stirrer plate.

Solutions were mixed with 8 mL of sodium acetate buffer (1.2 M, pH 3.8) while continuing the stirring. Then 1 mL of amyloglucosidase (300 units / mL) was added to each tube and mixed well using a magnetic stirrer. The tubes were kept in a water bath (50 °C) for 30 min. The contents of tubes were quantitatively transferred to 100 mL volumetric flasks using distilled water until the volume reached up to 100 mL. Aliquots of the solution were centrifuged at 1500 g for 10 min and 0.1 mL of solution was transferred to a test tube. GOD POD reagent 3.0 mL was mixed with the solution and incubated at 50 °C for 10 min. The absorbance values were measured at 510 nm against the reagent blank. Resistant starch content (g/ 100 g of sample) was calculated according to the following equation; Resistant starch =  $\Delta A \times F \times (10.3/0.1) \times (1/1000)$ x (100/W) x (162/180),  $\Delta A$  = averaged absorbance read against the reagent blank, F = conversion factor from absorbance to micrograms (For GODPOD reaction F = 100), W = Weight of the test portion analyzed. The results were expressed as the g / 100 g porridge (dm).

#### Ethical approval for the human studies

Informed written consent was obtained from each subject participating in the three studies, namely determination of GI and GL of porridges, and determination of short-term and long-term efficacies of FMP consumption on healthy adults before recruiting to the study. Ethical approval was obtained from the Ethics Review Committee of Faculty of Livestock, Fisheries and Nutrition, Wayamba University of Sri Lanka (201509HI01).

#### Determination of GI and GL of porridges

Glycemic indices of all FMP were determined with 10 healthy adults as a cross over study. The general information of the participants were presented in Table 1. After an overnight fasting for 8 to 10 h a capillary blood sample was obtained and blood glucose level was measured using a glucometer and test strips (Prodigy no-code glucometer and test strips, Prodigy Diabetes Care,

LLC (Charlotte, NC, USA). Glucose was used as the standard reference. Subjects were given 25 g of glucose (GSK, Glaxco Welcome, Ceylon Ltd., Sri Lanka) in 250 mL of water and postprandial blood glucose concentrations were measured at 15, 30, 45, 60, 90 and 120 min after ingestion of glucose. Incremental area under the curve (IUAC) was calculated using a graph constructed with blood glucose concentration vs. time. The above procedure was repeated three separate days for every subject and the mean IUAC was calculated for control. FMP containing 25 g of digestible carbohydrate was given to fasted subjects on separate days and blood glucose level was measured at fasting, 15, 30, 45, 60, 90 and 120 min. IUAC was calculated for each porridge type and the percentage ratio of IUAC for porridge and average IUAC for glucose were taken as GI for each individual. The GI values of each subject were averaged to obtain the final GI values. Glycemic load (GL) was estimated by multiplying the GI by the number of net carbohydrates in a given serving (25 g). FMP demonstrated the highest phenolic content, antioxidant activities and low GI and GL values were used to determine the short term and long term efficacy of FMP consumption.

#### Determination of efficacy of FMP consumption Subjects

Two independent studies were conducted to determine the short term and long term efficacies of FMP consumption. Subjects for both studies were recruited separately by open advertisements and were free from any known chronic disease conditions. General information of subjects participating in each study are presented in Table 1. At the screening visit which was 1 month before the study fasting blood glucose level, blood pressure, and anthropometry were assessed. They were instructed 2 weeks before the commencement of the interventions to avoid consumption of any finger millet based foods otherwise to keep to their normal diet except the FMP provided by the investigators to the completion of the trial period.

 Table 1 General information about participants involved in different studies

	Determination of glycemic index and glycemic load values of FMP $(n = 10)$	Determination of effects of short-term effi- cacy on consumption of FMP $(n = 10)$	Determination of effects of long-term effi- cacy on consumption of FMP $(n = 18)$
Age (y)	24.5 ± 1.33	34 ± 9	45.6 ± 5.6
Body weight (kg)	60.75 ± 8.94	52.0 ± 10.5	60.3 ± 10.3
BMI (kg / m <sup>-2</sup> )	22.01 ± 1.95	22.34 ± 4.30	22.5 ± 2.6
Waist circumference (cm)	77.79 ± 6.49	83.5 ± 6.78	84.1 ± 6.9
Waist to hip ratio	$0.9 \pm 0.5$	$0.9 \pm 0.4$	$0.9 \pm 0.1$
Systolic blood pressure (mmHg)	118.0 ± 8.5	119 ± 10.6	117.9 ± 12.2
Diastolic blood pressure (mmHg)	76 ± 7	78 ± 5	71.9 ± 8.6

# Determination of short- term efficacy of FMP consumption

#### Protocol

Ten subjects (4 men and 6 women) participated in the short-term efficacy study. Each subject ingested 200 mL of FMP after 12 h fasting. Heparinized blood (5 mL) was taken by venipuncture at baseline (after fasting) and at 1, 2, 3 and 4 h after ingesting FMP with vacuum tubes (so-dium fluoride). Plasma was separated by low-speed centrifugation (1500 g at 4 °C for 10 min) in a refrigerated centrifuge (3-18R TOMOS Life Science Group, Belmont, MA, USA) and stored at - 80 °C until analysis.

### Determination of long term efficacy of FMP consumption *Protocol*

The protocol followed here was a randomized controlled cross over study. Entry into the treatment arm or the control arm was randomized by stratification to ensure balance of the two arms. Eighteen subjects (8 men and 12 women) were recruited for the 8 wk. FMP intervention followed by an 8 wk. control period or the reverse sequence. Treatment periods were separated by a 4 wk. washout period in which subjects returned to the usual diet. After washout period subjects crossed over to the other arm of the study. Treatment group was provided with 200 mL of freshly prepared FMP as their mid-morning meal between 10.00 am to 10.30 am for 3 days per week for 8wk. The control group was kept to their normal dietary regime. Dietary recalls (24 h) were collected from the subjects 1 day per week during the study period and nutrient intake was analyzed using Food Base 2000 software (Institute of Brain Chemistry and Human Nutrition, University of North London, UK) which was updated by the Department of Applied Nutrition, Wayamba University of Sri Lanka including Sri Lankan food composition data.

Blood samples were collected from 12 h fasted subjects by venipuncture on day 1(baseline) and day 57 (end). Blood samples were drawn separately for plasma and serum and they were analyzed for plasma blood glucose level, plasma antioxidant power and serum total cholesterol level. Blood glucose levels were determined spectrophotometrically using a commercial kit (Glucose GOD-PAP liquid ready for use, BIOLABO, Maizy, France). Plasma glucose values were calculated using the given standard concentration and expressed as mg /dL. Total cholesterol levels were determined using enzymatic colorimetric determination (Stanbio cholesterol Liqui Colour Procedure No 1010, STANBIO) and were calculated using the given standard concentration and expressed as mg/dL.

#### Plasma antioxidant capacity assays

Two methods were employed to measure the antioxidant capacity of plasma, namely the ferric ion reducing antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC) assays.

The FRAP values of blood samples were determined by the method explained by (Benzie and Strain 1996). The FRAP value was measured at 593 nm, 4 min after 30  $\mu$ L of plasma was added to 1 mL of Fe (III)-TPTZ by which time the reaction was completed at 37 °C. Standard curve with the concentration ranged from 0.1–1 mM / L was prepared from a stock solution (0.278 g FeSO<sub>4</sub>.7H<sub>2</sub>O to 1 L of distilled H<sub>2</sub>O) and used for the calculations. The results are expressed as  $\mu$ mol L<sup>-1</sup> of antioxidant power.

The TEAC assay described by (Re et al. 1999) was used with minor modifications. The ABTS. solution was prepared by mixing 2.5 mM AAPH with 2.0 mM ABTS in 100 mM saline phosphate buffer (pH 7.4, 0.15 M NaCl) (PBS). The solution was kept in a water bath at 60 °C for 16 min, covered with aluminum foil to protect from light. The prepared ABTS. solution was filtered using mediumporosity filter paper before being mixed with the plasma sample. A blank was used for each measurement to account for the decrease in the absorbance of the radical solution with time. The TEAC was measured by mixing 40 µL of the plasma sample with 1960 µL of the ABTS. solution. The absorbance value of the reaction mixture was read at 734 nm immediately at the point of mixing  $(t_0)$  and after 6 min  $(t_6)$ . The decrease in absorbance at 734 nm after 6 min of addition of trolox and extract was calculated using the following equation:  $\Delta A$  trolox = (At<sub>0</sub>, trolox - At<sub>6</sub>, trolox) - (At<sub>0</sub>, blank - At<sub>6</sub>, blank), where  $\Delta A$ is the reduction of absorbance and A the absorbance at a given time. TEAC values were expressed as micromoles of trolox equivalents (TE) per  $\mu$ mol L<sup>-1</sup>.

#### Statistical analysis

All *in vitro* experiments were conducted in triplicates and data were reported as mean ± standard deviation. The differences of mean values among 12 finger millet porridges were determined by one way analysis of variance (ANOVA) followed by Turkey's Honestly Significant Difference (HSD) multiple rank tests at  $p \le 0.05$ , significance level. In the efficacy studies the differences of group means were analyzed by paired *t*- test with 95% CI. All statistical analysis was performed by SPSS version 16.

#### **Results and discussion**

#### Total phenolic content (TPC)

The TPC values of soluble phenolic extracts of FMP are presented in Table 2. The TPC of different FMP preparations varied from 24.6 to 44.7  $\mu$ mol FAE /g (dm). In the present study, roasting of raw finger millet flour significantly increased the TPC of soluble phenolic extracts of FMP preparations compared to those prepared using unroasted flour. In agreement with present findings, Gavirangappa and Krishnapura (2014) showed that

 Table 2 Phenolic content, antioxidant activities and glycemic response of porridges

			Porridge category	TPC*	DRSA*	GI*	GI category*	GL*	GL category*
Raw	Small*	Roasted	FMP1	$38.40 \pm 1.84^{a}$	$20.01 \pm 0.86^{a}$	$69\pm09^{a}$	Medium	17.25	Medium
		Unroasted	FMP2	$31.13 \pm 1.35^{b}$	$19.32 \pm 2.16^{a}$	$52 \pm 16^{b}$	Low	13	Medium
	Large*	Roasted	FMP3	$37.89 \pm 0.81^{a}$	$18.99 \pm 0.13^{a}$	$85 \pm 10^{\circ}$	High	21.25	High
		Unroasted	FMP4	$24.62 \pm 5.15^{\circ}$	$16.65 \pm 1.78^{\circ}$	$41 \pm 21^{d}$	Low	10.25	Low
Parboiled (15 min steaming)	Small	Roasted	FMP5	$44.67 \pm 0.51^{d}$	$27.52 \pm 0.88^{d}$	$48 \pm 28^{d}$	Low	12	Medium
		Unroasted	FMP6	$44.62 \pm 0.16^{d}$	$23.47 \pm 1.20^{e}$	$39 \pm 11^{d}$	Low	9.75	Low
	Large	Roasted	FMP7	$44.46\pm0.38^d$	$21.09 \pm 0.87^{a}$	$45 \pm 18^{d}$	Low	11.25	Medium
		Unroasted	FMP8	$43.12 \pm 1.25^{d}$	$20.03 \pm 0.75^{a}$	$45 \pm 13^{d}$	Low	11.25	Medium
Parboiled (30 min steaming)	Small	Roasted	FMP9	$30.45 \pm 0.54^{b}$	$19.58 \pm 0.84^{a}$	$38\pm08^{d}$	Low	9.5	Low
		Unroasted	FMP10	$29.24 \pm 1.19^{b}$	$19.35 \pm 0.80^{a}$	$45 \pm 19^{d}$	Low	11.25	Medium
	Large	Roasted	FMP11	$31.13 \pm 1.35^{b}$	15.79 ± 1.36 <sup>c</sup>	$38 \pm 18^{d}$	Low	9.5	Low
		Unroasted	FMP12	$29.53 \pm 3.73^{b}$	$16.83 \pm 0.89^{\circ}$	$48 \pm 11^{d}$	Low	12	Medium

\*TPC Total Phenolic content, DRSA 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability, GI Glycemic index, GL Glycemic load, Small- 0.038 mm; Large – 0.18 mm; Low GI value < 55, Medium GI value 69–56, High GI value > 70; Low GL value < 10, Medium GL value + 11–19, High GL value > 20; Same letters in each column are not significantly different (> 0.05)

roasting of finger millet grains increased the TPC by 17%. Further, Infante et al. (2010) reported increased TPC of barley due to roasting. The increment of TPC could be associated with the release of bound phenolic compounds upon the breakdown of cellular constituents (Boateng et al. 2008). However, in the present work significant differences of TPC were not observed for FMP prepared using roasted and unroasted flour of parboiled millet grains (Table 2). TPC of FMP which used raw flour showed a significant difference between the two particle sizes. However, TPC of FMP prepared with flour of parboiled millet grains did not vary significantly between the two particle sizes.

The soluble phenolic extracts of FMP (FMP5-FMP8) prepared using 15 min parboiled finger millet grain flour showed higher TPC compared to the porridges prepared using raw and 30 min parboiled finger millet grains. Parboiling process involved steeping the grains for equilibrium moisture content, steaming or boiling to gelatinize the starch and finally dehydrating the grains to remove the moisture to a safe level appropriate for storage. Previous studies have shown an optimum steaming time of 30 min for finger millets (Dharmaraj et al. 2011). In the present study two time periods (15 and 30 min) were used for the steaming of grains. The results showed that finger millet grains parboiled using 15 min steaming had higher TPC compared to that of 30 min steaming of grains. In accordance with these results, Bora et al. (2018) showed that parboiling increased the free phenolic content of pearl and proso millets. In addition, porridge prepared using parboiled pearl millet had three times higher free phenolic content compared to that of porridge prepared with parboiled proso millets (Bora et al. 2018). These results elaborated the fact that irrespective of the processing method inherent phenolic content of the millet verity retained. Furthermore, authors explained that the parboiling is an efficient way to increase the free and bound phenolic acid contents in millet products and increment of free phenolic contents may be due to the migration of the free phenolic compounds from the outer layers of the grain into the endosperm. In addition, release of bound phenolic compounds during the parboiling process may increase TPC of soluble phenolics (Bora et al. 2018). In a previous study Kato et al. (1983) reported the increased free phenolic content in parboiled rice. However, Yeo and Shahidi (2015) reported that interaction of the released phenolics from insoluble bound fraction was not equally accounted for the soluble fraction due to their possible interaction with the proteins in lentil seeds.

#### DPPH radical scavenging activity (DRSA)

The DRSA of FMP in this study ranged from 15.8 to 27.5  $\mu$ mol TE / g (dm) (Table 2). FMP prepared using 15 min parboiled finger millet grains showed a higher DRSA compared to those of raw and 30 min parboiled grains. Generally, FMP prepared using roasted finger millet flour showed a higher DRSA than their unroasted counter parts. According to Pushparaj and Urooj (2014), roasting significantly increased the antioxidant activity of pearl millet. Pradeep and Guha (2011) showed increased radical scavenging activity in little millet upon roasting. This could be due to release of bound phenolic compounds as well as possible transformation of some compound to smaller active molecules (Boateng et al. 2008).

#### Resistant starch (RS)

The term RS is defined as the sum of starch and products of starch degradation which are not absorbed in the small intestine of healthy individuals. RS provides fermentable carbohydrates for colonic bacteria. The content of RS is negatively correlated with GI and helps to reduce the postprandial glycemic responses, levels of cholesterol and triacylglycerol (Tharanathan and Mahadevamma 2003). In the present work, the content of RS of representative FMP ranged from 23.21 to 25.40 g/ 100 g of porridge (Table 3). According to previous studies finger millet starch had high crystallinity and required high temperature to gelatinize compared to rice (Mohan et al. 2005). Singhe and Ali (2006) showed that finger millet starch is more difficult to hydrolyze in vitro by fungal ă-amylase. Furthermore, Roopa and Premavalli (2008) reported the effect of processing methods on starch fractions of finger millets. They showed that roasting and pressure cooking increased the RS fractions in finger millets compared to their raw counterparts. However, results of the present study did not show significant differences of RS content among the tested FMP.

#### **Glycemic response**

Glycemic Index (GI) is defined as the incremental area under the curve (AUC) for blood glucose response after ingestion of a food relative to that produced by a reference food (white bread or glucose) given in an equivalent carbohydrate content (50 or 25 g) taken by the same subject over a specified period of time (Jenkins et al. 1981). GI of foods is categorized as low (GI value < 55), medium (GI value 56–69) or high GI foods (> 70). Glycemic load (GL) is a measure of both quality (GI value) and quantity (grams per serving) of carbohydrate in a meal. GL is determined by multiplying its GI value by the amount of carbohydrate in the food in each serving (Brand-Miller et al. 2009).

GI and GL values of finger millet porridges were presented in Table 2. All FMP exhibited low GI values (< 55) except that prepared with the raw roasted flour which showed high GI value (> 70) for large particle size (FMP3) and medium GI value (56–69) for small particle size (FMP1). Generally, in the present study selection of two particle sizes did not significantly affect the glycemic response of porridges. Previous studies exhibited low GI values for different food preparations of millet grains (Arora and Srivastava 2002; Jayasinghe et al. 2013; Surekha et al. 2013). According to Jayasinghe et al. (2013) foods prepared with finger millet flour with larger particle size showed low GI. Inconsistency of the results of present study with previous work could be due to the use of different particle sizes of flour and foods with different cooking methods. Foods, *Rotti* and *Pittu* were prepared by Jayasinghe et al. (2013) with industrial milled finger millet flour (smaller particle size) and stone ground flour (larger particle size). Furthermore, they have used dry heating and steaming for *Rotti* and *Pittu* preparation, respectively whereas FMP was prepared using open boiling with excess of water in our study.

The present work demonstrated that roasting of finger millet flour increased the GI of prepared porridges. It was also observed that FMP of parboiled grains had low GI compared to those prepared with raw grains. Parboiling is a hydrothermal treatment that includes soaking in water, heating, drying and milling used for paddy in general. It improved the milling quality, nutrient availability; organoleptic properties, and resistant starch content. During the parboiling process, the crystalline structure of the starch present in rice is transformed into an amorphous form (Hanny et al. 2015). Starch digestion in foods, and consequent glycemic responses are altered by hydrothemal treatments, as well as different processing methods employed (Rashmi and Urooj 2003; Jaisut et al. 2008). Parbolied rice has reduced the glycemic responses up to 30% compared to raw rice (Larsen et al. 2000; Widowati et al. 2010) In another study barnyard millet grains were subjecting to heat treatment (60 °C) and intermittent cooling cycles for 1 h to prepare Uppma (Ugare et al. 2014). In agreement with the present work, they showed that GI of Uppma prepared with processed barnyard millets was low compared to that of unprocessed grain meal.

## Determination of short term efficacy of FMP on plasma antioxidant power

The FMP5 prepared using parboiled finger millet (15 min steamed), flour (small, roasted) was ingested by subjects and plasma antioxidant capacity (PAC) measured by FRAP and TEAC assays (Fig. 1). Figure 1a presents the PAC of subjects as determined by FRAP. The PAC of subjects at baseline was 745.65  $\mu$ mol Fe (II) eq/L and increased to 771.29 at 1 h, 846.93 at 2 h, 791.46 at 3 h and 773. 27  $\mu$ mol Fe (II) eq/L at 4 h. The increment of

 Table 3 Resistant starch content of representative finger millet porridges

		5 1 5		
Processing applied to grain	Particle size of flour	Processing applied to flour	Porridge category	Resistant starch content (g/ 100 g)
Raw	Small	Roasted	FMP1	$25.40 \pm 1.50^{a}$
		Unroasted	FMP2	$23.24 \pm 1.06^{a}$
Parboiled (with 15 min steaming)	Small	Roasted	FMP5	$23.21 \pm 2.12^{a}$
		Unroasted	FMP6	$24.89 \pm 2.56^{a}$
Parboiled (with 30 min steaming)	Small	Unroasted	FMP10	$23.71 \pm 2.23^{a}$

Entries carrying the same letters in each column are not significantly different (p > 0.05)

PAC was significant (p < 0.05) at 1, 2, 3 and 4 h after ingestion of FMP and were 3.4, 13.6, 6.1 and 3.7%, respectively. The mean plasma antioxidant capacity of subjects after ingesting FMP increased as determined by TEAC to 350.24, 409.11, and 395.59, respectively at 1, 2 and 3 h from 332.9 µmol TE /L at 0 h (Fig. 1b). The increments of antioxidant capacity were 5.2, 22.9 and 18.8% at 1, 2, and 3 h respectively, compared to the baseline. The increment of PAC after ingestion of FMP, as determined by TEAC, was not significant (p > 0.05). The results from both analytical methods suggest that PAC exerted from of FMP consumption is sustained at least for 3 h.

The increase of plasma antioxidant power after the consumption of foods and beverages rich in phenolic compounds has been observed in previous studies (Scalbert et al. 2005; Torabian et al. 2009). Torabian et al. (2009) showed a significant increase of PAC as measured by FRAP following the intake of walnut and almond meal for a period of 210 min. According to previous studies a peak value of PAC was achieved between 30 and 60 min after ingestion of phenolic rich foods except for olive oil, which resulted in a peak PAC at approximately 120 min post ingestion (Torabian et al. 2009). In the present study, PAC measured by both TEAC and

FRAP assays reached a peak value at 2 h after consumption of FMP. The TPC of plasma after ingestion of porridges was not measured in the present study. Therefore, the increased PAC could be an additive effect of different antioxidants compounds found in FMP which provides several vitamins, minerals and non-nutrient antioxidants. Therefore, additional studies which measure concentrations of plasma total phenolic and urinary metabolites along with PAC are needed to understand the kinetics of antioxidant efficacy of phenolic compounds present in FMP.

### Determination of long term efficacy of FMP consumption on blood parameters

Table 4 presents the nutrient intake of subjects during FMP feeding trial and control which shows that no significant differences existed for any of the nutrients concerned. In the present study, fasting plasma glucose and total cholesterol levels were not significantly different after the consumption of FMP for 8 weeks (Table 5). However a previous study showed a significant reduction in both fasting and postprandial blood glucose levels in patients with non-insulin dependent diabetes after consumption of millet incorporated foods (Geetha and Parvathi 1990). Ugare



**Table 4** Mean dietary intake of subjects during intervention and control

	Intervention	Control
Energy (Cal)	1736 ± 53	1748 ± 28
Carbohydrates (g)	285 ± 14	278 ± 10
Protein (g)	40 ± 5	38 ± 5
Fat (g)	48 ± 6	49 ± 2
Calcium (mg)	648 ± 10	646 ± 5
Magnesium (mg)	443 ± 14	440 ± 10
Iron (mg)	22 ± 2	21 ± 2
Zinc (mg)	10.3 ± 1.0	10 ± 2
Copper (mg)	2.49 ± 0.2	2.90 ± 0.1
Selenium (µg)	131.7 ± 0.5	128.5 ± 1.0
lodine (µg)	53 ± 2	54 ± 1
Thiamin (mg)	1.63 ± 0.30	1.70 ± 0.10
Riboflavin (mg)	0.82 ± 0.02	$0.90 \pm 0.01$
Niacin eq (mg)	60.8 ± 2.3	58.0 ± 1.2
Vitamin B6 (mg)	2.19 ± 0.30	2.25 ± 0.20
Vitamin B12 (µg)	0.19 ± 0.01	$0.12 \pm 0.05$
Folate (µg)	427.9 ± 6.0	429.1 ± 5.0
Vitamin C (mg)	73.8 ± 2.4	72.9 ± 1

No significant differences was observed among the mean dietary intake of subjects during intervention and control periods; Values are mean  $\pm$  SD (n = 18)

et al. (2014) conducted a feeding intervention with foods prepared using barnyard millet on non-diabetic subjects for 28 days. The subjects cooked the daily dose of test meal in the form of Uppma or rice and consumed in breakfast, lunch and / or dinner. According to their results the mean initial fasting blood glucose level of the experimental group was 103.24 mg / dL which was reduced to 96.06 mg / dL after the feeding intervention, indicating statistically significant (p < 0.01) reduction compared to the control group. Furthermore, the decrease in the total cholesterol level from an initial value of 217.66 to 215.37 mg / dL was observed

(Ugare et al. 2014). Feeding intervention conducted by Surekha et al. (2013) showed that the feeding of barnyard millet based health mix on healthy volunteers for 28 days reduced blood glucose and total cholesterol levels by 7 and 8%, respectively. In the present study long term FMP consumption has significantly (p < 0.05) improved the PAC of subjects and was measured by TEAC and FRAP assays (Table 5). FRAP of subjects improved by 12.4% whereas TEAC improved by 45.1% at the end of FMP intervention. In a previous study, Vanhethof et al. (1997) reported a small but significant increment in total plasma antioxidant capacity as measured by TEAC after consumption of 6 cups of green tea per day for 4 weeks.

In the present study FMP were freshly prepared and served as a mid-morning snack to the subjects. Table 4 shows that there were no significant differences among the macro and micro nutrient intakes between periods of intervention and control which they consumed their normal mid-morning snacks. Furthermore, fasting plasma glucose and total cholesterol values were not significantly different after the consumption of FMP during intervention period. Therefore, continuation of this study for extended time period or the future studies with patients who are having diabetes or high blood cholesterol levels may be beneficial to see the effect of consumption of FMP on blood glucose and cholesterol levels.

#### Conclusion

The overall results of the present study indicated that finger millet porridge is a nutritious meal with several health benefits. Parboiling of finger millet grains for 15 min increased the phenolic contents and antioxidant activities and improved the glycemic response in vivo. The short- and long-term consumption of finger millet porridges improved the plasma total antioxidant capacity of healthy adults. Further research is warranted to examine the potential of finger

**Table 5** Long term efficacy on consumption of finger millet porridge on plasma glucose, serum total cholesterol and plasma antioxidant levels of healthy adults

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Parameter	Baseline	End of control	Baseline	End of treatment
Fasting plasma glucose (mg/dL)	80 ± 10	87 ± 9*	87 ± 11	83 ± 13
Total cholesterol (mg/dL)	164 ± 13	175 ± 34	161 ± 22	177 ± 35
Plasma antioxidant capacity				
Ferric ion reducing antioxidant power <sup>a</sup>	718.3 ± 140.0	695.3 ± 99.9	679.5 ± 120.3	763.9 ± 105.3*
Trolox equivalent antioxidant capacity <sup>b</sup>	332.3 ± 116.2	269.1 ± 88.4	296.9 ± 122.8	431.0 ± 55.4*

All values are mean  $\pm$  SD, n = 18

\*Significantly different from the baseline value p < 0.05 (paired t test)

<sup>a</sup>µmoles of Fe (II) equivalents per L

<sup>b</sup>µmoles of trolox equivalents per L

## millet porridge on improving the antioxidant capacity of patients with diabetes and metabolic syndrome.

#### Abbreviations

AAPH-2,2: Azobis (2-methylpropionamidine) dihydrochloride; ABTS-2,2: Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; DPPH-2,2: Diphenyl-1-picrylhydrazyl; DRSA: DPPH radical scavenging activity; FAE: Ferulic acid equivalent; FMP: Finger millet porridges; FRAP: Ferric ion reducing antioxidant power; GAE: Gallic acid equivalent; GI: Glycemic index; GL: Glycemic load; IUAC: Incremental area under the curve; PG: Plasma glucose; PAC: Plasma antioxidant capacity; TC: Total cholesterol; TE: Trolox equivalent; TEAC: Trolox equivalent antioxidant capacity

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#### Authors' contributions

DK designed experiments, collected and analyzed data, and prepared the draft of the manuscript. AC conceptualized the study, designed experiments, supervised the study, read and edited the manuscript. PA supervised the protocol of human studies. FS read and edited the manuscript. All authors read and approved the manuscript.

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#### Availability of data and materials

All data supporting this study are included in this manuscript. Further details are available upon request from the corresponding author.

#### **Competing interests**

Authors declare that they have no competing interests.

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