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Hypocholesterolemic and Hypoglycemic Effect of Cowpea (Vigna unguiculata L. Walp) Incorporated Experimental Diets in Wistar Rats (Rattus norvegicus)

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Abstract

This study was carried out to investigate the hypocholesterolemic and hypoglycemic effect of four cowpea cultivars. Wistar rats were grouped and fed a high fat diet with 20% Bombay (BO), 20% MI 35 (MI), 20% Waruni (WA), 20% Dawala (DA) in comparison with 20% casein (HFD). Serum total cholesterol, non-HDL cholesterol, triacylglyceride and glucose concentrations and serum antioxidant activity were analysed. Serum lipids, glucose concentrations and serum antioxidant activity in WA fed rats were significantly lower (P < 0.05) than HFD. Therefore, raw Waruni cowpea produced significant hypolipidemic and hypoglycemic effects in Wistar rats.

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Keywords: Cowpea; wistar rats; cholesterol; hypolipidemic effect; hypoglycemic effect

1. Introduction

Non Communicable Diseases are on the increase at present and poor dietary habits have been the main cause behind this. This phenomena started after the industrial revolution in 1760 and the human diet started changing from

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unrefined whole grains and vegetable with high fiber to refined grains with low fiber content and more animal products. Hyperlipidemic states especially hypercholesterolemia with reference to lipoprotein disorder and hyperglycemia and hyperinsulinemia has become a major cause for these diet related chronic diseases.

Consumption of legumes has been related to many beneficial physiological effects in controlling and preventing various metabolic diseases. Legumes are also rich in dietary fiber and carbohydrates (Rochfort and Panozzo, 2007) and lipids, polyphenols and bioactive components are present as minor components (Pastor-Cavada et al., 2009). Hypocholesterolemic and hypoglycemic ability of legumes may due to the presence of phytic acid, dietary fiber, saponins, phytosterols, proteins, peptides and their amino acid profiles (Reynold et al., 2006).

Hypolipdemic effect of legumes has been shown both in animal models and in clinical trials (Anderson, 1985; Hu et al., 2000). One cohort study showed that those who eat legumes 4 times a week had a risk of coronary heart disease (CHD) that was 21% lower than those who ate them less than once weekly (Bazzano et al., 2001). The cowpea (*Vigna unguiculata* L. Walp) is considered a grain legume or pulse which is potential agent of reducing serum lipids and glucose. There is a number of cowpea cultivars developed in Sri Lanka with different morphological features and it is important identify the functional properties of commonly consumed cultivars *in vivo* and *in vitro*. This is the first study which investigated the effect of commonly consumed Sri Lankan cowpea cultivars namely 'Dawala', 'Waruni', 'Bombay', and 'MI 35' on serum lipids, serum glucose and serum antioxidant activity using Wistar rats.

2. Materials and methods

2.1. Animals and diets

Male Wistar rats (7 weeks old) were purchased from the Medical Research Institute, Colombo, Sri Lanka. They were housed individually in wire meshed cages with free access to food and water. The animal facility was maintained on a 12 light/dark cycle at a temperature of $23 \pm 1^{\circ}$ C and relative humidity of $60 \pm 5^{\circ}$. The rats were randomly assigned into five groups (n=5) (Liyanage et al., 2007; Liyanage et al., 2008). Government Certified Cowpea cultivar seeds, 'Waruni', 'Bombay', 'Dawala', and 'MI 35' were purchased from government seed farm, Palwehera, Dambulla, Sri Lanka. Samples were identified and certified by the Agriculture officers of the government seed farm. Proximate composition of four cowpea cultivars was determined by the procedures of the Association of Official Analytical Chemists (1995) (Data not shown). According to proximate analysis data, Crude Fiber content in Dawala, Waruni, Bombay and MI 35 cowpea cultivars were 5.66%, 6.38%, 6.85% and 6.49% respectively. Experimental diets were prepared according to AIN 93G semi purified rodent diet (diet composition not shown). The experimental rats were fed for 6 weeks, with either 20% fat as a basal diet (HFD), and compared with 20% fat enriched diets containing 20% whole cowpea powder from cowpea cultivars (BO; WA; DA; and MI). There was no difference in food intake and final body weight among groups at the end of the experimental period. Blood samples (1 mL) were taken at the beginning and at the end of the six weeks period between 09.00 and 10.00 h from the jugular vein of fasting rats anesthetized with sodium pentobarbital. The samples were collected without any anticoagulant; and serum was separated by centrifugation at 1500 g for 20 min. This experimental design was approved by the Animal Experiment Committee of Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka. All animal procedures conformed to standard principles described in Guide for the Care and Use of Laboratory Animals (1985).

2.2. Serum lipid ,glucose and serum antioxidant activity estimation

Total cholesterol (TC), HDL-cholesterol (HDL-C), triglyceride (TG) and glucose concentrations in the serum (Table1) were determined enzymatically using commercially available reagent kits (ProDia Internationals, Germany).

Serum antioxidant activity was measured by Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain, 1996).

2.3. Statistical analysis

Completely Randomized Design (CRD) was conducted and data were analyzed by one-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS software programme (SAS Institute, Cary, NC,USA). Significant differences among means were separated by the Duncan's Multiple Rang test (DMRT). Differences at P < 0.05 were considered as significant.

3. Results

3.1. Serum lipids, serum antioxidant activity and serum glucose in rats fed experimental diets for 6 weeks

Table 1. The Serum Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Triacylglycerol concentrations (mmol/L) and serum antioxidant activity in rats fed experimental diets for 6 weeks.

Treatment	Total Cholesterol	HDL-Cholesterol	LDL-Cholesterol	Triacylglycerol	Serum antioxidant	Glucose
	(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)	activity (µmol/L)	(mmol/L)
BO	1.26±0.17 ^b	0.56±0.16 ^{ab}	0.42±0.13 ^b	1.37±0.39ª	464.44±19.32ª	1.25 ± 1.07^{ab}
MI	1.09±0.24b	0.72±0.22a	0.19±0.11d	0.90±0.11b	450.62±38.16a	$1.85\pm0.56a$
WA	0.89±0.30b	0.43±0.06b	0.37±0.05c	0.45±0.10c	472.89±23.52a	$0.46 \pm 1.15 b$
DA	1.28±0.37ab	0.57±0.17ab	0.47±0.34b	1.17±0.40ab	279.02±29.59c	1.36± 0.78ab
HFD	1.59±0.22a	0.53±0.11ab	0.87±0.14a	0.93±0.19b	386.12±34.32b	$1.82\pm0.80a$

* Values are expressed as means \pm sd. the values in each column with different superscripts a to d are significantly different (P < 0.05).

Table 1 shows the serum total cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C), Triacylglycerol concentrations (mmol/L), Glucose and antioxidant activity in rats fed experimental diets for 6 weeks.

The serum total cholesterol level was lower (P < 0.05) in rats fed BO, MI, and WA diets than that in the HFD fed rats. The serum LDL-C level was lower (P < 0.05) in all cowpea incorporated diets fed rats compared to HFD. Serum triacylglycerol level was lower (P < 0.05) in WA fed group compared to HFD fed group. Serum HDL-C level was higher (P < 0.05) in MI fed group compared to that in WA fed group. The serum Glucose concentration in the WA fed group was (P < 0.05) lower than the BO, MI, DA and HFD fed groups. Serum antioxidant activity was high (P < 0.05) in WA, BO and MI fed groups compared to that in HFD and DA fed groups.

4. Discussion

Results show that lower TC concentration (P < 0.05) in rats fed BO, MI, and WA diets compared to HFD was in agreement with lower LDL-C concentration compared to HFD and this results were in agreement with data reported for Hamsters fed whole cowpea and cowpea isolate in a previous study (Frota et al., 2008). Serum TC concentration in rats fed experimental diets correlate with serum LDL-C concentration, correlation coefficient being r = 0.430 (P < 0.05). Higher dietary fiber content in cowpeas may have increased fecal lipid excretion and reduced serum TC level as suggested by Bazzano et al. (2011).

Lower (P < 0.05) serum TC and LDL-C concentrations in WA, BO and MI cowpea diet fed rats were further supported by higher (P < 0.05) serum antioxidant activity (correlation coefficients being r = 0.42 and r = 0.82 (P < 0.05), respectively) compared to HFD, and this was in agreement with previous studies showing that lower serum lipid level may be due to higher antioxidant activities preventing lipid peroxidation (Jemai et al., 2008). Dark colored cowpea, Waruni may have higher antioxidant activity and phenol content modulated serum antioxidant activity (P < 0.05) and serum triacylglcerol level (P < 0.05) compared to same group of the samples (Beninger and Hosfield, 2003; Troszynska and Ciska, 2002).

Higher HDL-C level (P < 0.05) in MI fed group compared to that in HFD and WA diet may be supported by higher soluble fiber content in MI 35 compared to other cultivars and was in agreement with a previous study

(Pande et al., 2012). The impact of pectin, or soluble fibers, on lipid metabolism has been well established both in humans and animal models (Aller et al., 2004; Levrat et al., 1993). The hypocholesterolemic action of fiber is partly mediated by a lower absorption of intestinal bile acid, thus increasing fecal bile acid loss, and its synthesis in liver. The physicochemical properties of soluble fiber alter hepatic cholesterol and lipoprotein metabolism, resulting in the lowering of plasma LDL cholesterol. Fermentation of dietary fiber by the intestinal microflora could modify the short chain fatty acid production thereby reducing acetate and increasing propionate synthesis. This, in turn, reduces the endogenous synthesis of cholesterol, fatty acids and low density lipoproteins (Arranz, 2012).

The lower serum glucose levels in WA fed group could also be due to high dietary fiber content and high antioxidant capacity in Waruni cowpea powder over the other three cultivars. The interactions of other constituents in the grain legume can bind starch and affect blood glucose levels. High fiber content in cowpea has a high satiety effect and therefore can reduce the likelihood of excessive consumption of calories. Anti-nutrients found in cowpea including phyticacid, phenolics and saponins may also be responsible for lowering plasma glucose as observed for soy beans (Chang et al., 2008).

5. Conclusions

Waruni, MI 35 and Bombay cowpea cultivars incorporated high fat diets reduced serum total cholesterol and serum LDL-cholesterol concentration in rats compared with rats fed high fat control diet. Waruni cowpea cultivar with highest serum antioxidant activity reduced the serum triacylglycerol level as well as glucose concentration compared to HFD fed group. Therefore MI 35 and Bombay cowpea cultivars showed hypocholesterolemic effects while Waruni cultivar showed both hypolipidemic and hypoglycemic effects.

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