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Antioxidant and hypolipidaemic effects of a novel yam–boxthorn noodle in an in vivo murine model

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Abstract

Yam–boxthorn noodle, a newly developed functional noodle, was prepared by mixing wheat flour, yam and boxthorn for the purpose of lowering serum cholesterol and oxidation status in vivo. To determine whether yam–boxthorn noodle exhibits hypolipidaemic and antioxidant effects in vivo, we examined the changes in triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C) serum and oxidation levels in the serum and visceral organs of BALB/c females after continuously consuming the test diets for 5 weeks. The TG, TC, TG/HDL-C and TC/HDL-C serum levels in the experimental group decreased in a dose-dependent manner when the yam–boxthorn noodle concentration in the AIN 76 diet rose from 3 to 30%. However, the HDL-C and LDL-C serum levels did not significantly change. The TBARS oxidation index of the heart, liver and kidney significantly decreased compared with that of control group. The increase in tissue antioxidant capacity varied in magnitude: heart > liver > kidney. The total antioxidant status in the serum significantly increased in the 3% yam–boxthorn noodle diet experimental group. These experiments demonstrate that the functional noodle, yam–boxthorn noodle, exhibits hypolipidaemic and antioxidant effects in an in vivo murine model. Further, these results suggest that the functional ingredients in the yam and boxthorn, traditionally used as medicinal plants and functional foods, present greater health benefits than that of traditional noodles. These results will be important and useful for the future exploitation of traditional materials to develop a novel functional food for safeguarding health of hyperlipidaemia patients.

Keywords: Yam–boxthorn noodles; Hypolipidaemic effect; Antioxidant capacity; In vivo murine model

1. Introduction

In vivo oxidation products, namely free radicals and reactive oxygen species (ROS), have been associated with the etiology and/or progression of a number of diseases and ageing (Moskovitz, Yim, & Chock, 2002). Coronary risk factors, including hypercholesterolemia (especially total cholesterol and low density lipoprotein cholesterol), hyperlipidemia (e.g. triglyceride), age, hypertension, diabetes mellitus, and smoking, are associated with enhanced oxidative stress (Ide et al., 2002). Atherosclerosis is suggested to be linked to the oxidation of lipoproteins, primarily LDL, in the vascular wall (Sentman et al., 2001). However, total antioxidant capacity (TAC) is significantly reduced in stroke patients compared with controls (Gariballa, Hutchin, & Sinclair, 2002). It has proven that propofol (known to possess antioxidant activity) increases tissue antioxidant capacities (such as red blood cell, liver, kidney, heart, lung) during anesthesia (Runzer, Ansley, Godin, & Chambers, 2002). Clearly, hyperlipidemia (high in TC, TG, LDL-C) and in vivo oxidation stress are detrimental to health. However, hypolipidaemic

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and antioxidant effects can prevent diseases. Both rats and mice have been used to study atherosclerosis and exogenous cholesterol or steroid metabolism. Injection of poloxamer-407 (P-407), a hyperlipidaemic and atherogenesis inducer, into rats and mice causes significant hypercholesterolemia and hypertriglyceridemia in a dose-dependent manner (Palmer, Emeson, & Johnston, 1997). There is a 55% or higher homology in the amino acid sequence between the BALB/c mouse apolipoprotein A-II (apoA-II) and human apoA-II (Miller, Lee, LeBoeuf, & Shively, 1987), suggesting their similarities in lipoprotein cholesterol metabolism. In addition, mice and humans show similar changes in serum steroid following oophorectomy and orchiectomy (Brunner et al., 1986). These experiments may support the usefulness of murine models for the study of hypolipidaemic effects in vivo.

Noodles are popular and one of the most important staple foods in many countries around the world. Traditional noodles are made from simple ingredients, namely, wheat flour, water, salt, and alkali (Baik & Lee, 2003). However, nutritional compositions having health benefits in noodles have seldom been discussed. Even though the consumption of functional foods increases yearly, noodles for hyperlipidaemic patients have not yet been developed and evaluated. However, some ingredients or materials may potentially enhance hypolipidaemic functions in traditional foods, such as cereals and their products. In recent years, sorghum flour fermentation has been used as a functional ingredient in composite breads, particularly in the sourdough bread making process (Hugo, Rooney, & Taylor, 2003). A valuable oil, phytosterol, from wet-milled corn fibre and grain sorghum, has been reported to lower serum LDL-cholesterol levels (Singh et al., 2003; Singh, Moreau, & Hicks, 2003). Cereal brans, such as rice bran, oat bran, dehulled barley and β-glucan-enriched barley, have also demonstrated relatively high bile acid binding values in vitro, possibly related to their insoluble dietary fibre (IDF) or protein anionic, cationic, physical and chemical structure, composition, metabolites, or their interaction with binding sites (Kahlon & Woodruff, 2003). The bile acid binding hypothesis involves increased fecal excretion, regarded as a possible dietary fibre mechanism for lowering cholesterol (Lund, Gee, Brown, Wood, & Johnson, 1989). One of the Taiwanese yams, *Dioscorea japonica* Thunb var. pseudojaponica Yamamoto, consistently improves the cholesterol profile in the plasma and liver of adult BALB/c mice fed a 50% yam diet (Chen, Wang, Chang, & Wang, 2003). Some functional steroidal saponins existing in yams have been isolated and identified (Du, Liu, Fu, Xia, & Xia, 2002; Yang, Lu, & Hwang, 2003; Yin et al., 2003). The anti-hypercholesterolemia saponin mechanism in yams is probably related to its inhibitory activity against cholesterol absorption (Ma et al., 2002).

Oxidative stress, an imbalance between oxidant production and antioxidant defences in favour of the former, has been shown to be involved in the atherogenesis process (Ide et al., 2002), ageing (Moskovitz et al., 2002), cholestatic liver disease (Ljubuncic, Tanne, & Bomzon, 2000), cancer, neurodegenerative, and cardiovascular diseases (Squier, 2001). It has been found that oxidative stress processes in connection with continued systemic inflammatory response syndrome (SIRS) may promote the development of multiple organ failure (MOF) (Motoyama et al., 2003). Oxidation and antioxidant balance in the body is a crucial factor in the pathophysiology of various diseases (Basu & Eriksson, 2000). It was found that circulatory vitamin E, an antioxidant, may surge plasma isoprostanes and prostaglandins produced by the oxidant-antioxidant imbalance (Basu & Eriksson, 2000). The storage protein and mucilage of yam tubers (*Dioscorea batatas*) may play roles as antiradicals and antioxidants (Hou, Hsu, & Lee, 2002; Hou et al., 2001). Dried wolfberries (*Lycium barbarum*), also called boxthorn or matrimony-vine, fruits of a Chinese medicinal plant, increase the superoxide dismutase (SOD), catalase (CAT) and total antioxidant capacity in experimental mice (Li, Yang, Ren, & Wang, 2002). The glycoconjugates of these plants can inhibit low density lipoprotein (LDL) peroxidation (Huang, Tian, Wang, Dong, & Wu, 2001). Total flavonoids from *L. barbarum* L. show the scavenging effect on active oxygen radicals and inhibit the heat output from PMA-stimulated PMN (polymorphonuclear leukocyte) and L1210 cells (Huang, Tan, Shen, & Lu, 1998). They also have protective effects on lipid peroxidation in liver mitochondria and red blood cells in rats (Huang, Lu, Shen, & Lu, 1999).

From the researches mentioned above, some natural materials, especially yam and boxthorn, may have hypolipidaemic and antioxidant effects that prevent coronary diseases. A novel functional product, yam–boxthorn noodle, for the purpose of lowering serum cholesterol and oxidation status in vivo, was developed to meet the needs of desirable functional characteristics. The aims of this study were to examine both the hypolipidaemic and antioxidant effects of yam–boxthorn noodles, a newly developed noodle enriched with yam (*Dioscorea alate* L.) and boxthorn (*L. barbarum*), on female BALB/c mice through a five week feeding trial in an in vivo murine model.

2. Materials and methods

2.1. Sample preparation and nutrition analysis

The yam–boxthorn noodle, a newly developed noodle in Taiwan, was enriched with yam (*D. alate* L.) and boxthorn (*L. barbarum*). A new variety of *Dioscorea alata* L.

cv. Tainong No. 2 was provided by the Hualien District Agricultural Research and Extension Station, Council of Agriculture. The fresh yam was washed with tap water, the skin pared, cut into slices, lyophilized, milled with a Roter Speed Mill (model: RT-02A) into powder and then passed through a 200 mesh sieve. Semi-dried boxthorn, a medicinal plant fruit, was purchased from a Chinese traditional medicine store, lyophilized, milled into powder and passed through a 200 mesh sieve. The patent flour (11.9% protein and 13.3% moisture (Liou, 2003)) and salt were obtained from a local supermarket. The yam–boxthorn noodle was prepared from the patent flour, yam powder, boxthorn powder, sodium chloride and water in 600, 330, 70, 20 and 320 g proportions, respectively (Liou, 2003). The flour, powders and salted water were mixed to form dough. The dough was allowed to stand for 1 h after mixing, then passed through a noodle roller machine with a 3-mm gap. The dough was then folded and passed through sheeting rollers, six times. The dough sheet was allowed to stand for 1 h and then put through the sheeting rollers at progressively smaller gap settings, finally to 1.40 mm. The sheet was cut into strips of about 30 cm in length. The yam–boxthorn noodles were then placed in plastic bags and stored at 4 °C for 24 h prior to use (Baik & Lee, 2003; Liou, 2003). The moisture, protein, mineral (ash), lipid and crude fibre contents of the yam–boxthorn noodles were 30.7, 10.3, 2.27, 0.15, and 19.7%, respectively. The moisture, protein, and mineral (ash) content of the yam–boxthorn noodles were determined according to Approved Methods 44-15A, 46-30, and 08-01 (AACC, 2000; Park, Hong, & Baik, 2003). The carbohydrate content of the yam–boxthorn noodle, calculated using the equation: 100- (moisture + protein + mineral + lipid + crude fibre)%, was 36.9%. The protein, mineral, lipid, crude fibre, and carbohydrate content of the yam–boxthorn noodle, based on the dry weight, were 14.9%, 3.28%, 0.22%, 28.5%, and 53.2%, respectively.

2.2. Experimental animals and diets

The BALB/c mice (female, 6 weeks old) were obtained from the National Laboratory Animal Center, National Applied Research Laboratories, National Science Council in Taipei, ROC and maintained in the Department of Food Science at National Chung Hsing University College of Agriculture and Natural Resources in Taichung, Taiwan, ROC. The mice were housed and kept on a chow diet (lab standard diet) for 2 weeks before feeding the experimental diet. The animal room was kept on a 12-h-light and 12-h-dark cycle with constant temperature (25 ± 2 °C) and humidity. After this equilibrium period, the mice were divided into four groups ($n = 9$) and each group was fed with one of the specified experimental diets for five consecutive weeks ad libitum. Mice food intake or body weight were measured every 3 days during the study period. There were no differences in food intake, feed efficiency and body weight gain between the four diet groups. The specified diets consisted of AIN 76 semi-purified diets that were partially replaced, respectively, by 0%, 3%, 12%, and 30% dry yam–boxthorn noodle powder. The ingredients and experimental feed chemical analyses are given in Table 1. The contents included casein, corn starch, cellulose, soybean oil, and mineral mix in AIN 76 formula adjusted by the protein, carbohydrate, crude fibre, lipid, and ash contents of the dry noodle powder to maintain the nutritional balance in the specified AIN 76 experimental feeds. According to the chemical analysis, the 3% and 12% yam–boxthorn noodle feed mixes had similar nutrition compositions to the AIN 76 formula.

2.3. Analytical methods

2.3.1. Serum lipid and lipoprotein analysis

On day 35, after 14 h of fasting, the animals were anaesthetized with ether and blood was collected using retro-orbital venous plexus puncture into a 1.5-ml vial. The mice were then sacrificed using CO_2 inhalation. The blood was allowed to stand for 2 h at room temperature, then centrifuged at 12,000*g* for 15 min at 4 °C to separate the serum. Serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and low density lipoproteins (LDL-C) were analyzed using a colorimetric method with a RANDOX assay Kit Cat. No. TR 213, CH 201, CH 203, and CH 1350, respectively.

2.3.2. Oxidation status (TBARS) of visceral organs

An end product, malondialdehyde (MDA), of polygenic fatty acid peroxidative decomposition in the lipid peroxidation process may accumulate in the tissues. This is indicative of the oxidation stress extent in vivo (Ljubuncic et al., 2000). Tissue MDA was determined using the thiobarbituric acid reactive substances (TBARS) method. TBARS formation in the tissues was measured using fluorometric assay, as described previously (Ide et al., 2002). In brief, multiple female BALB/c mice visceral organs were collected, including heart, lung, liver, spleen, and kidney, and homogenized in cold 0.01 M potassium phosphate buffer to a final concentration of 25% (w/v). These homogenates were used to determine the TBARS values. The chemicals used in this study were purchased from Sigma Chemical Co. One ml of the tissue homogenate was first added and mixed well with 1 ml of 10% (w/v) trichloroacetic acid (TCA), and centrifuged at 1,500*g* for 10 min to precipitate the protein. One ml of the supernatants or the standards (1,1,3,3-tetra-methoxy propane, TMP) were added and mixed well with 1 ml of 0.4% (w/v) thiobarbituric acid (TBA), and 0.1 ml of 0.2% (w/v) butylated hydroxy

Table 1
Ingredients and chemical analyses of the experimental feeds

Ingredients (g/kg feed)	Percentage of yam–boxthorn noodle in AIN 76 diet			
	0%	3%	12%	30%
Casein	200	195.5	182.2	155.4
DL-methionine	3	3	3	3
Corn starch	325	309	261.2	165.5
Sucrose	325	325	325	325
Cellulose	50	41.5	15.8	–
Soybean oil	50	49.9	49.7	49.3
AIN76 mineral mix	35	34.02	31.06	25.16
AIN76 vitamin mix	10	10	10	10
Choline bitartrate	2	2	2	2
Dry Noodle powder[a]	–	30	120	300
Chemical analysis[b](g/kg feed)				
Carbohydrate	650	650	650	628
Crude protein	200	200	200	194
Crude fat	50	50	50	48.3
Ash	35	35	35	33.8
Crude fibre	50	50	50	82.5
Gross energy (kcal/kg feed)	3850	3850	3850	3723

[a] Dry noodle powder was obtained from fresh yam–boxthorn noodle air-dried in oven (50 °C) overnight, and contained 14.9% crude protein, 3.28% ash, 0.22% crude fat, 28.5% crude fibre and 53.2% carbohydrate.

[b] Calculation was based on the yam–boxthorn noodle dry powder composition.

toluene (BHT). After incubation in a water bath at 50 °C for 1 h, the samples were added and mixed well with 2 ml of isobutanol (2-methyl-propanol), and then centrifuged at 1500*g* for 10 min. The supernatant was measured fluorometrically at an excitation wavelength of 515 nm and emission wavelength of 550 nm (Hitachi F-2000 spectrophotometer). The TBARS tissue levels were expressed as MDA equivalents (e.g. nmol MDA/mg wet tissue) using 1,1,3,3-tetra-methoxy propane as the standard.

2.3.3. Total serum antioxidant status (TAS)

The total serum antioxidant capacity was analyzed by a colorimetric method using the RANDOX assay Kit Cat. No. NX 2332. The assay principle is briefly described as follows: ABTS (2,2′-azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and hydrogen peroxide to produce the radical cation ABTS$^{\cdot+}$. This compound has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample (i.e. serum) cause suppression of this colour production to a degree that is proportion to their antioxidant concentration or activity. The 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, at a concentration of 2.5 mmol/l was used as the standard in this kit. Aliquots of 20 μL reagent blank, standard, and the sample were, respectively, pipetted into a cuvette, mixed well with 1 ml chromogen (metmyoglobin, ABTS) and the initial absorbance then read at 600 nm at 37 °C (A_1). After reacting for exactly 3 min, the absorbance (A_2) at the same wavelength was read. $A_2 - A_1 = \Delta A$ of sample/standard/blank. The total antioxidant capacity (TAC) was expressed as mmol/l and calculated using the equation: $\text{mmol/l} = \text{factor} \times (\Delta A\ \text{blank} - \Delta A\ \text{sample})$. $\text{Factor} = \text{conc of standard}/(\Delta A\ \text{blank} - \Delta A\ \text{sample})$.

2.4. Statistical analysis

The effects of yam–boxthorn noodle diet effect were assayed using the unpaired Student's *t*-test with one tail to test the significant differences from the control group. Values are expressed as group averages and SD ($n = 9$). Differences were considered statistically significant if $P = \leqslant 0.05$. The significant differences were labelled according to the P value as: $^{*}P = \leqslant 0.05$; $^{\#}P = \leqslant 0.01$; $^{\S}P = \leqslant 0.005$.

3. Results

3.1. Serum lipids profile

The serum triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) concentrations of the mice fed the yam–boxthorn noodle diets are given in Table 2. The serum triglyceride concentrations were significantly decreased ($P < 0.05$) in the yam–boxthorn noodle-fed mice at all experimental concentrations used. The mice fed the yam–boxthorn noodle diet at the concentration of 30% had significantly lower levels of total cholesterol than had the mice fed the AIN76 diet ($P = 0.046$). Both serum high density lipoprotein choles-

Table 2
Yam–boxthorn noodle diet effects on serum lipids of female BALB/c mice through 5 weeks feeding

Content[a] of serum lipid[b]	Percentage of yam–boxthorn noodle in AIN 76 diet			
	0%	3%	12%	30%
TG (mg/dl)	226 ± 90.9	139 ± 106[*] ($P = 0.045$)[c]	161 ± 53.7[*] ($P = 0.050$)	123 ± 40.9[§] ($P = 0.005$)
TC (mg/dl)	122 ± 9.70	128 ± 24.8($P = 0.231$)	117 ± 8.60 ($P = 0.151$)	112 ± 11.6[*] ($P = 0.046$)
HDL-C (mg/dl)	92.2 ± 6.37	101 ± 19.6 ($P = 0.116$)	92.0 ± 3.49($P = 0.461$)	89.2 ± 8.48 ($P = 0.200$)
LDL-C (mg/dl)	18.8 ± 6.17	19.2 ± 7.56 ($P = 0.457$)	17.6 ± 5.36 ($P = 0.345$)	16.4 ± 5.17 ($P = 0.207$)
TG/HDL-C (mg/mg)	2.46 ± 1.02	1.48 ± 1.15[*] ($P = 0.041$)	1.73 ± 0.56[*] ($P = 0.046$)	1.39 ± 0.44[#] ($P = 0.008$)
TC/HDL-C (mg/mg)	1.32 ± 0.07	1.27 ± 0.10 ($P = 0.145$)	1.25 ± 0.04[*]($P = 0.013$)	1.26 ± 0.05[*] ($P = 0.028$)
TC/LDL-C (mg/mg)	6.17 ± 1.25	7.48 ± 2.65 ($P = 0.132$)	7.02 ± 1.56 ($P = 0.129$)	7.43 ± 2.25 ($P = 0.104$)
HDL/LDL (mg/mg)	4.70 ± 1.11	6.03 ± 2.44 ($P = 0.111$)	5.58 ± 1.36 ($P = 0.096$)	5.94 ± 1.95 ($P = 0.079$)

[a] Data are expressed as averages ± SD ($n = 9$).

[b] TG = triglyceride; TC = total cholesterol; HDL-C = high density lipoprotein cholesterol; LDL-C = low density lipoprotein cholesterol.

[c] Mean values were significantly different from the control group (0% yam–boxthorn noodle diet) shown (unpaired Student's *t*-test with one tail).

[*] $P \leqslant 0.05$.

[#] $P \leqslant 0.01$.

[§] $P \leqslant 0.005$.

terol and low density lipoprotein cholesterol concentrations decreased slightly as the added yam–boxthorn noodle concentration in the diet increased. However, this decrease in serum high density lipoprotein cholesterol and low density lipoprotein cholesterol was still not prominent ($P > 0.05$) at the 30% yam–boxthorn noodle diet concentration. To understand the TG, TC, HDL-C and LDL-C interaction, the TG/HDL-C, TC/HDL-C, TC/LDL-C and HDL/LDL serum ratios of the mice were calculated. It was found that the mice fed 3%, 12% and 30% yam–boxthorn noodle diets had significantly lower levels of TG/HDL-C ratios. The mice fed 12% and 30% yam–boxthorn noodle diets had significantly lower levels of TC/HDL-C ratios. Both the TC/LDL-C and HDL/LDL ratios in all mice fed yam–boxthorn noodle diets did not change significantly ($P > 0.05$) during the experimental period.

3.2. Oxidation status (TBARS) of different visceral organs

To evaluate the lipid peroxidation status, the TBARS of the tissue homogenates from different visceral organs from the mice fed 0%, 3%, 12% and 30%, respectively, yam–boxthorn noodle diets were determined. The TBARS concentrations in different visceral organs from the female BALB/c mice are given in Table 3. The TBARS levels of heart homogenates were 2.10 ± 0.787, 1.14 ± 0.387, 1.14 ± 0.225, and 0.865 ± 0.287 nmol/mg wet tissue, respectively, in the mice fed 0, 3, 12 and 30% yam–boxthorn noodle diets. The TBARS levels of heart from the mice fed 3%, 12% and 30% yam–boxthorn noodle diets significantly decreased ($P < 0.05$), and there was trend of decrease in TBARS, as the percentage of yam–boxthorn noodle increased. The TBARS concentrations of lung homogenates were 16.1 ± 2.13, 17.0 ± 2.12, 17.1 ± 1.39, and 16.1 ± 2.78 nmol/mg wet tissue, respectively, in the mice fed 0, 3, 12 and 30% yam–boxthorn noodle diets. There were no significant differences in TBARS levels between the four groups. The TBARS concentrations for the liver homogenates were 1.28 ± 0.342, 0.935 ± 0.333, 0.815 ± 0.270, and 0.680 ± 0.234 nmol/mg wet tissue, respectively, in the mice fed 0%, 3%, 12% and 30% yam–boxthorn noodle diets. The TBARS levels for the livers from mice fed 3%, 12% and 30% yam–boxthorn noodle diets significantly decreased ($P < 0.05$) in a dose-dependent manner. The TBARS concentrations of spleen homogenates were 10.8 ± 2.46, 15.0 ± 3.88, 15.0 ± 4.223, and 12.56 ± 1.79 nmol/mg wet tissue, respectively, in mice fed 0%, 3%, 12% and 30% yam–boxthorn noodle diets. The spleen TBARS levels from mice fed 3% and 12% yam–boxthorn noodle diets significantly increased ($P < 0.05$). The TBARS concentrations of kidney homogenates were 11.4 ± 2.04, 13.8 ± 4.63, 8.65 ± 1.55, and 6.97 ± 2.86 nmol/mg wet tissue, respectively, in mice fed 0%, 3%, 12% and 30% yam–boxthorn noodle diets. The kidney TBARS levels from mice fed 12% and 30% yam–boxthorn noodle diets significantly decreased ($P < 0.05$) in a dose-dependent manner.

3.3. Total serum antioxidant status

The total serum antioxidant status (TAS) measurement may be regarded as more physiologically representative than the individual antioxidants in the tissues. This is believed to be a useful evaluation of how much the present antioxidants can protect against oxidative damage to membranes and other cellular components (Gariballa et al., 2002). The serum TAS of mice fed the yam–boxthorn noodle diets are given in Fig. 1. The serum TAS of mice fed the yam–boxthorn noodle diet at the 3% concentration had significantly higher levels compared with the mice fed the AIN76 diet ($P = 0.003$). However, the serum TAS, at both the

Table 3
Yam–boxthorn noodle diet effects on TBARS concentrations in different visceral organs of female BALB/c mice through 5 weeks feeding

TBARS[a] (nmol MDA /mg wet tissue)	percentage of yam–boxthorn noodle in AIN 76 diet			
	0%	3%	12%	30%
Heart	2.10 ± 0.787	1.14 ± 0.387[§] ($P = 0.0034$)[b]	1.14 ± 0.225[§] ($P = 0.0014$)	0.865 ± 0.287[§] ($P = 0.0002$)
Lung	16.1 ± 2.130	17.0 ± 2.120 ($P = 0.2037$)	17.1 ± 1.39 ($P = 0.1428$)	16.1 ± 2.78 ($P = 0.4911$)
Liver	1.28 ± 0.342	0.935 ± 0.333[*] ($P = 0.0258$)	0.815 ± 0.270[§] ($P = 0.0027$)	0.680 ± 0.234[§] ($P = 0.0002$)
Spleen	10.8 ± 2.457	15.0 ± 3.884[*] ($P = 0.0109$)	15.0 ± 4.23[*] ($P = 0.0138$)	12.6 ± 1.79 ($P = 0.0542$)
Kidney	11.4 ± 2.036	13.8 ± 4.626 ($P = 0.0910$)	8.65 ± 1.547[#] ($P = 0.0026$)	6.97 ± 2.86[§] ($P = 0.0008$)

[a] Data are expressed as averages ± SD ($n = 9$).
[b] Mean values were significantly different from the control group (0% yam–boxthorn noodle diet) shown (unpaired Student's t-test with one tail).
[*] $P \leqslant 0.05$.
[#] $P \leqslant 0.01$.
[§] $P \leqslant 0.005$.

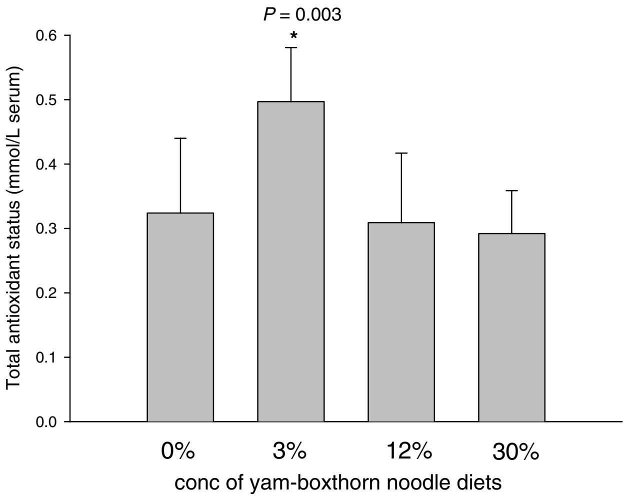

Fig. 1. Effects of yam–boxthorn noodle diets on total antioxidant status of serum from female BALB/c mice through 5 weeks feeding: (a) data are expressed as averages ± SD ($n = 9$); (b) mean values were significantly different from the control group (0% yam–boxthorn noodle diet) shown (unpaired Student's t-test with one tail): $^{*}P \leqslant 0.05$.

12% and 30% yam–boxthorn noodle diet concentrations, did not change significantly ($P > 0.05$).

4. Disscussion

The results of this study show that yam–boxthorn noodle, a newly developed noodle enriched with yam (*D. alate* L.) and boxthorn (*L. barbarum*), is capable of decreasing serum lipids and increasing the total antioxidant capacities of the serum and tissues (including heart, liver, kidney) in an in vivo mouse model. Serum lipids, including TG and TC, were decreased significantly by feeding the mice with yam–boxthorn noodle diets for 5 weeks (Table 2). The hypolipidaemic effects may be attributable to the ability of the yam constituents (*D. alate* L.) (Chen et al., 2003; Du et al., 2002; Ma et al., 2002; Yang et al., 2003; Yin et al., 2003). As expected, treatment with yam–boxthorn noodle diets at adequate concentrations substantially decreased the serum triglyceride and total cholesterol levels. Contrary to our hypothesis, the HDL-C and LDL-C serum concentrations were slightly decreased. However, the HDL/LDL ratios in the experimental groups increased slightly compared with the control group. This finding means that yam–boxthorn noodle diets decreased the LDL-C levels more than the HDL-C levels. Clearly, a decrease in TC, TG, LDL-C (bad cholesterol) serum levels with an increase in HDL-C (good cholesterol) in vivo is favourable to good health. We have shown that the yam–boxthorn noodle diet can regulate serum lipid profiles. Previous studies suggested that yam–boxthorn noodle, a functional noodle, may act as a staple food with hypolipidaemic effects.

Serum lipids in the BALB/c mice were decreased by the yam–boxthorn noodle diets. Total antioxidant capacities were increased in vivo. The total antioxidant capacities can be evaluated by the reduction of the TBARS level or increase of the total antioxidant status (TAS). This study demonstrated the natural relative TBARS responses in the visceral tissues to be: lung (16.1 ± 2.13 nmol MDA/mg wet tissue) > kidney (11.4 ± 2.04 nmol MDA/mg wet tissue) > spleen (10.8 ± 2.46 nmol MDA/mg wet tissue) > heart (2.10 ± 0.787 nmol MDA/mg wet tissue) > liver (1.28 ± 0.342 nmol MDA/mg wet tissue) (Table 3). The maximum TBARS level in the lung may be explained by its hyperoxic environment, resulting from continuous oxygen inhalation from the air. The minimum TBARS level in the liver may be explained by its powerful detoxifying enzyme system in preventing excess oxidation. The oxidative stress in the visceral tissues in vivo seems variable. This finding might also be explained by differences in the inherent susceptibility of each tissue to lipid peroxidation (Runzer et al., 2002). Our study in BALB/c mice demonstrates that continuously feeding yam–boxthorn noodle diets can reduce the TBARS levels in the heart, liver, and kidney (Table 3). The antioxidant capacities of the visceral tissues were variably affected by the yam–boxthorn noodle diets. At the 30% yam–boxthorn noodle diets, the decreases in heart, liver, and kidney TBARS levels were 58.9%, 47.0%, and 38.9%, respectively. The relative tissue responses to the protective effects of the yam–boxthorn noodle diets were: heart > liver > kidney. Because of the hyperoxic environment, the yam–boxthorn noodle diets may not reverse the oxidative stress in the lungs. This finding may be explained by the differences of protective effects in visceral tissues against lipid peroxidation by the yam–boxthorn noodle constituents. De La Cruz, Sedeno, Carmona, & Sanchez de la Cuesta (1998) made an observation, in an in vitro study of the effects of propofol (a free radical scavenger) on the forced peroxidation of various rat tissues. They found that the relative tissue responses to the protective effects of propofol were: heart > lung > kidney > liver. Runzer et al. (2002) made a similar observation in an in vivo study on the effects of propofol on various tissues. However, they found the relative tissue responses were: liver > kidney > heart > lung. This discrepancy was explained by differences in regional blood flow to individual organs during the in vivo phase, which might affect the amount of drug accumulating in the tissues (Runzer et al., 2002). Our results also demonstrated that increases in tissue antioxidant capacities by yam–boxthorn noodle diets, varied in their magnitude but were dissimilar to the propofol values in a rat model. These findings imply that the varying antioxidant components existing in yam–boxthorn noodles or other foods may have different target organ priorities. Both yam and boxthorn, existing in yam–boxthorn noodles, were proven to be antioxidants (Hou et al., 2002; Hou et al., 2001; Huang et al., 2001; Huang et al., 1998; Li et al., 2002). However, the TBARS levels in spleen were increased by yam–boxthorn noodle diets. We found that yam–boxthorn noodle diets could increase immune responses in vivo (data not shown). We propose one possible explanation for TBARS increases in the spleen by yam–boxthorn noodle diets: the secondary lymphoid organ (e.g. spleen) is enhanced by yam–boxthorn noodle diets, especially in a process known as the respiratory burst, resulting in hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-), and nitric oxide (NO), which are directly toxic to bacteria.

The serum total antioxidant status (TAS) result was significantly increased by the yam–boxthorn noodle diets at only 3% concentration (Fig. 1). However, at higher yam–boxthorn noodle diet concentrations, TAS was not increased significantly in the serum. The total antioxidant status in the serum is an overall expression against oxidation in vivo and may be affected by many factors, such as the total levels of antioxidants and oxidants (Gariballa et al., 2002). These findings suggest that a 3% yam–boxthorn noodle diet might have maximum total antioxidant capacity for BALB/c mice for long-term feeding.

The hypolipidaemic and antioxidant effects of yam–boxthorn noodle diets may be attributable to the yam components (*D. alate* L.) (Chen et al., 2003; Du et al., 2002; Hou et al., 2002; Hou et al., 2001; Ma et al., 2002; Yang et al., 2003; Yin et al., 2003) and boxthorn (*L. barbarum*) (Huang et al., 2001; Huang et al., 1999; Huang et al., 1998; Li et al., 2002), e.g. saponins, polysaccharides and total flavonoids. Previous studies support our claim that yam–boxthorn noodle, a functional noodle, exhibits hypolipidaemic and antioxidant effects. However, the effects of compounds in yam–boxthorn noodles on organ function and health remain to be elucidated.

5. Conclusion

This study showed that yam–boxthorn noodle diets significantly decreased the triglyceride (TG) and total cholesterol (TC) levels in serum and increased the antioxidant capacities in both the serum and individual organs, especially in the heart, kidney and liver, in mice. The increases in tissue antioxidant capacities varied in magnitude as follows: heart > liver > kidney. These results will be important and useful for the future exploitation of yam and boxthorn to develop a novel functional food for safeguarding health of hyperlipidaemic patients.

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