Mal J Nutr 23(3): 449 - 460, 2017

Potential Hypocholesterolemic Activity of Flour from Leaves of Moringa (*Moringa oleifera L.*)

Indah Asrifah[1], Teti Estiasih[2*] & Hidayat Sujuti[3]

[1] *Master Program of Agricultural Product Technology, Faculty of Agricultural Technology, Brawijaya University*
[2] *Department of Food Science and Technology, Brawijaya University Jalan Veteran, Malang 65145, Indonesia*
[3] *Laboratory of Biochemistry Faculty of Medicine, Brawijaya University, Jalan Veteran, Malang 65145, Indonesia*

ABSTRACT

Introduction: Moringa (*Moringa oleifera* L.) leaves contain phytosterols and dietary fibres which may be beneficial in controlling blood cholesterol levels. This study was aimed at assessing the hypocholesterolemic effect of flour from leaves of *M. oleifera* L. (MLF) with white and red stalk in rats. **Methods:** Thirty male rats were divided into 6 groups, comprising a normal group (negative control), a hypercholestrolemic group (positive control) both of which were without MLF feeding, and 4 hypercholesterolemic groups fed MLF for 4 weeks in the following manner: (i) 0.822 mg/g bw/d white stalk (WM); (ii) 0.822 mg/g bw/d red stalk (RM); (iii) 0.02 ml/g bw/d commercial plant stanol ester (FS); and (iv) 0.001 mg/g bw/d ezetimibe (ET). At the end, serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-c), viscosity and pH of digesta, faecal cholesterol, and short chain fatty acids (SCFAs) were analysed. **Results**: TC levels in the WM, RM, FS and ET groups decreased by 42.0, 48.8, 48.4 and 52.8% respectively, compared to initial levels. The four groups also showed decreases in serum LDL-c levels by 30.3, 39.2, 37.9 and 46.7% respectively, over the feeding period. Faecal cholesterol levels of WM and RM were higher (63.93±1.87 and 90.11±1.77 mg/100 g faeces, respectively) than that of the positive control (51.30±4.03 mg/100 g) after 4 weeks. **Conclusion:** Flour from moringa leaves of white and red stalk trees showed potential hypocholesterolemic activity in rats.

Key words: Fecal cholesterol, hypercholesterolemia, moringa, phytosterols

INTRODUCTION

Cardiovascular diseases are a leading cause of death globally (WHO, 2015). Hypercholesterolemia, as one of the most important risk factors for development of cardiovascular diseases, is a lipoprotein metabolism disorder that is characterised by high serum total and low density lipoprotein cholesterol levels (Otunola *et al*., 2010). Moringa (*Moringa oleifera* L.) leaves contain phytosterols and dietary fibres which might be beneficial to control cholesterol levels. Rajanandh *et al.* (2012) reported that hydro-alcoholic extract of moringa leaves contains β-sitosterol at a concentration of 90 mg/g. Phytosterols are plant sterols that have a chemical structure similar to cholesterol. They competitively inhibit cholesterol absorption, thereby reducing total cholesterol (TC) and LDL

Correspondence: Teiti Estiasih; Email: *teties@ub.ac.id, teties@yahoo.co.id*

cholesterol (LDL-c) levels (Wang *et al*., 2015).

Previous studies have reported that dietary fibres, especially soluble dietary fibres, can reduce TC and LDL-c levels by trapping cholesterol in the viscous matrix, thereby eliminating more cholesterol into the large intestine (Kristensen *et al.* 2012). Unabsorbed dietary fibres are fermented in the colon and produce short-chain fatty acids (SCFAs) which have cholesterol lowering effects by inhibiting cholesterol synthesis (Wong *et al*., 2006).

Based on the colour of stalk, there are two type of moringa in Indonesia, white and red stalk (Figure 1). White moringa (WM) has green stalk and bigger leaves, meanwhile red moringa (RM) has red stalk and smaller leaves. Although moringa leaves have been reported to improve lipid profiles in animal and human studies, there is no study known which compares the hypocholesterolemic effects of white and red stalk moringa leaves *in vivo.* This study aimed at comparing the hypocholesterolemic effects of WM and RM moringa leaves flour in vivo, and also to compare their respective dietary fibres and phytosterol contents.

METHODS

Materials

WM and RM leaves of *M. oleifera* L.) were collected from Probolinggo, East Java, Indonesia. Reference standards of physterols (stigmasterol, campesterol, and β-sitosterol) for high performance liquid chromatography (HPLC) were obtained from Sigma-Aldrich, Singapore. Ezetimibe 10 mg was purchased as Ezetrol®. A commercial fermented dairy product that contains plant stanol esters (1.7 g/100 ml) was used as a comparison. Plant stanols are derivatives of phytosterols that are obtained by hydrogenation (Moreau, Whitaker & Hicks, 2002) and esterification (Weber, Weitkamp & Mukherjee, 2002). All other chemicals and solvents were of analytical grade and were purchased from Merck.

Preparation of moringa leaves flour

The moringa leaves flour preparation was carried out according to the method described by Kiranawati *et al*. (2012). This process consisted of several stages, that is, sorting, washing, blanching (steam blanching for 5 min at 97°C) and drying (in a cabinet dryer for 2 h at 40°C). The dried leaves were milled into fine powder using an electric blender and the flour was sieved through 100-mesh sieve. The moringa leaves flour was then analysed for proximate analysis, dietary fibres, and phytosterols (*β*-sitosterol, campesterol, and stigmasterol).

Determination of proximate composition

The sample of WM and RM moringa leaves flour were analysed for moisture, protein, fat, and ash contents by using the method of AOAC (2005), while carbohydrate content was analysed by difference.

Dietary fibre and phytosterols analysis

Analysis of dietary fibres was based on the method of Asp *et al*. (1983). Phytosterols (β-sitosterol, campesterol, and stigmasterol) analysis was according to Slavin & Yu (2012) with slight modifications. As much as 100 mg of moringa leaves flour was put into 15 ml glass tube, added with 3 ml of ethanol and 0.1% ascorbic acid (w/v). The mixture was shaken, mixed vigorously on a vortex mixer for 10 s, and then heated in a water bath shaker at 85°C for 5 min. Then 0.19 ml of 10 M KOH was added to the flour, shaken on a vortex mixer for 10 s, incubated in a water bath shaker at 85°C for 10 min, then immediately cooled on ice. Afterward, 3 ml of 1 M NaCl was added and the mixture was gently shaken five times. Subsequently, 3 ml of hexane was added, the mixture was shaken on a vortex mixer for 10 s, and centrifuged at 1000 rpm at

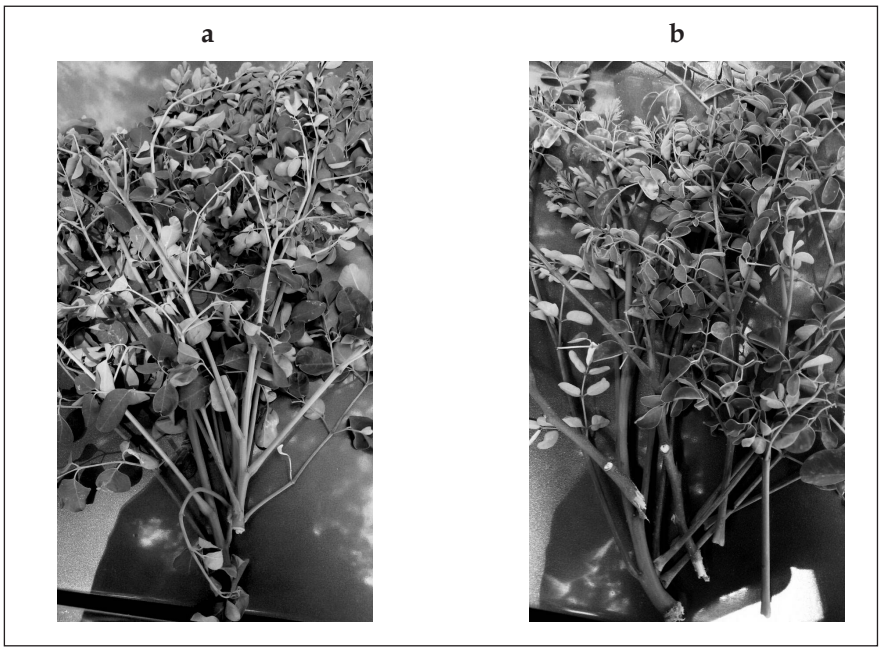

Figure 1. White (a) and red (b) stalk of *Moringa oleifera* tree

4°C for 5 min to obtain the supernatant. Hexane extraction was repeated twice. The supernatant was washed with 5 ml of 5% Na_2CO_3 (w/v), and then centrifuged at 1000 rpm at 4°C for 5 min. The supernatant was poured into a new tube and then washed with 5 ml ultrapure water. The supernatant was removed after gently shaking five times manually. The final supernatant was evaporated in a rotary vacuum evaporator. The resulting evaporated supernatant was dissolved in 0.25 ml isopropyl alcohol. Measurement of phytosterols concentration was done by using high performance liquid chromatography (HPLC) (Shimadzu LC 20AD, Kyoto, Japan; Column ODS 250 x 4.6 mm; flow rate 1 ml/min; methanol/ aquabidest 99.1:0.9 v/v as the mobile phase).

Animals and diets

All procedures performed in this study were approved by the Ethics Committee for Animal Studies of Brawijaya University (No. KEP-471-UB). Thirty male Wistar rats aged 2-3 months were randomly divided into 6 groups (n=5), namely negative control (received standard diet only), positive control (untreated hypercholesterolemic rats), hypercholesterolemic rats + white stalk moringa leaves flour (WM), hypercholesterolemic rats + red stalk moringa leaves flour (RM), hypercholesterolemic rats + commercial plant stanol esters containing fermented dairy product (FS), and hypercholesterolemic rats + ezetimibe (ezetrol) (ET). Each group was fed with standard AIN-93M *ad libitum*. After a week of acclimatisation, rats were induced into a hypercholesterolemia condition by force feeding pure cholesterol (1%), cholic acid (0.2%), quail egg yolk (7%), and beef fat (15%) for a week. After achieving a hypercholesterolemia condition (serum TC level >200 mg/dl), the rats were treated with WM moringa leaves flour 0.822 mg/g bw/d, RM moringa leaves flour 0.822 mg/g bw/d, commercial plant stanol ester containing fermented dairy product 0.02 ml/g bw/d, and

ezetimibe (ezetrol) 0.001 mg/g bw/d for 4 weeks by forced feeding. At the end of the intervention periods, serum TC and LDL-c levels, viscosity of small intestinal digesta, faecal cholesterol concentration, SCFAs concentrations, and pH of caecal digesta were measured. Extent of TC reduction after 4 weeks of feeding was measured by comparing the TC level at week 4 to the initial TC level before treatment of each group.

Determination of small intestinal digesta viscosity

Measurement of viscosity of small intestinal digesta was according to the method described by Passos *et al*. (2015). Viscosity of jejunal and ileal digesta was measured with a viscometer (Brookfield Digital Viscometer, Model DV-II+ Pro, Brookfield Engineering Laboratories Inc., Stoughton, MA).

Determination of faecal cholesterol concentration

Fecal samples were collected each day for 3 days before the intervention period ended from all groups (3 rats per group). Faecal cholesterol concentration was determined by the Liebermann-Burchard method (Plummer, 1977).

Determination of short-chain fatty acids (SCFAs) concentrations and pH in caecal digesta

After euthanasia with ether inhalation in the animal cage, the caecum was immediately removed and its content was collected to measure SCFAs concentration and pH. The caecal content was centrifuged at 10,000 rpm for 10 min. As much as 1 ml supernatant was mixed with 0.25 ml metaphosphoric acid. SCFAs concentration (acetate, propionate, and butyrate) was determined in the supernatant by using Gas Chromatography Mass Spectroscopy (GC-MS) (Shimadzu GCMS-QP2010S, Kyoto, Japan; equipped with a RTX-5MS column, helium carrier gas with a flow rate of 80 mL/min, using the FID detector). This procedure was according to Zhou & Hylemon (2014). The pH value of caecal content was measured by diluting the supernatant ten times by distilled water and measuring the pH by digital pH meter (Senz pHmeter, Trans Instruments, Singapore).

Statistical analysis

Results were presented as the mean ± standard deviation, and comparison between means of all groups were analysed by one-way analysis of variance (ANOVA) followed by the Tukey HSD test. Differences in mean values between nutrient contents of WM and RM moringa leaves flour were analysed using independent samples *t*-test. For all tests, $P < 0.05$ was considered as statistically significant. The statistical analyses were performed with SPSS version 22.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS

Proximate composition of fresh and flour of moringa leaves

The proximate composition of fresh moringa leaves and moringa leaves flour for both moringa types are presented in Table 1. The results showed that protein content of WM moringa leaves flour was significantly higher than of RM moringa leaves flour. The protein content of moringa leaves flour of both types of moringa was 25.91% to 27.25%, indicating that they are a good source of protein. The average content of ash of RM moringa leaves flour was significantly higher than of WM moringa leaves flour. However, fat and carbohydrate contents were similar ($P > 0.05$).

Dietary fibres and phytosterol content of moringa leaves flour

Table 1 shows that total dietary fibers content of WM and RM moringa leaves flour were 49.21% and 52.10% of dry basis.

Table 1. Nutritional composition of fresh moringa leaves and moringa leaves flour

Component (%, db)[1]	*White stalk moringa tree (WM)*		*Red stalk moringa tree (RM)*	
	Fresh leaves	*Leaves flour*	*Fresh leaves*	*Leaves flour*
Moisture	73.96 ± 0.11*	8.44 ± 0.09*	77.02 ± 0.10*	7.53 ± 0.14*
Crude protein	7.29 ± 0.15	27.25 ± 0.13*	6.31 ± 0.23	25.91 ± 0.16*
Crude fat	0.98 ± 0.25	7.06 ± 0.24	0.87 ± 0.16	7.02 ± 0.11
Ash	2.30 ± 0.08	6.12 ± 0.05*	2.57 ± 0.04	7.05 ± 0.09*
Carbohydrate	15.48 ± 0.21*	51.14 ± 0.51	13.24 ± 0.33*	52.50 ± 0.28
Dietary fibers		49.21 ± 0.41	52.10 ± 0.70	
- Insoluble dietary fibres		39.97 ± 0.20	41.77 ± 0.43	
- Soluble dietary fibres		9.24 ± 0.21	10.33 ± 0.27	

1 = value are mean ± SD (n = 2).
db = dry basis (g/g).
* = the means are significantly different at the 5% level by independent samples t-test.

Table 2. Bioactive compounds of moringa leaves flour

Bioactive compounds (ppm)	*White stalk moringa leaves flour*	*Red stalk moringa leaves flour*
Phytosterols	723.49	252.84
- β-sitosterol	616.13	35.47
- Campesterol	100.56	211.35
- Stigmasterol	6.80	6.02

β-sitosterol was the major phytosterol in WM moringa leaves flour, followed by campesterol and stigmasterol. Meanwhile, the main component of phytosterols content in RM moringa leaves flour was campesterol, followed by β-sitosterol and stigmasterol (Table 2).

Viscosity of small intestinal digesta and fecal cholesterol excretion

The main purpose of small intestinal digesta viscosity measurement was to know one of the mechanisms of cholesterol-lowering effects by cholesterol absorption inhibition. No statistical differences were found in the viscosity of small intestinal digesta among all groups ($P > 0.05$; Table 3). The faecal cholesterol excretion at the end of intervention periods was significantly different among all groups ($P < 0.05$; Table 3). Higher faecal cholesterol concentrations were found in treated groups compared to untreated group. Faecal cholesterol concentrations of WM and RM groups were higher than of PC group. Meanwhile, the RM group had significantly higher faecal cholesterol concentration than WM and FS groups, but lower than ET group.

SCFAs concentrations and pH of caecal digesta

There was a significant difference in SCFAs concentration among the six groups ($P < 0.05$; Table 4). SCFAs concentration was lower in the PC group than in both NC group and treated groups (Table 4). We found significantly higher SCFA concentrations in the RM group compared

Table 3. The viscosity of small intestinal digesta and faecal cholesterol concentration

Group	*Viscosity of small intestinal digesta (cP)*	*Fecal cholesterol concentration (mg/100 g faeces)*
NC	2.33 ± 0.06[a]	130.46 ± 2.79[f]
PC	2.32 ± 0.11[a]	51.30 ± 4.03[a]
WM	2.56 ± 0.15[a]	63.93 ± 1.87[b]
RM	2.63 ± 0.17[a]	90.11 ± 1.67[d]
FS	2.46 ± 0.19[a]	79.94 ± 3.23[c]
ET	2.41 ± 0.05[a]	101.51 ± 3.28[e]

Values (mean ± SD, n = 3) in the same column with different letters indicated significant difference (ANOVA followed by Tukey HSD, $P < 0.05$).
NC negative control, PC positive control, WM hypercholesterolemia rats + WM moringa leaves flour, RM hypercholesterolemic rats + RM moringa leaves flour, FS hypercholesterolemia rats + commercial plant stanol esters containing fermented dairy product, ET hypercholesterolemia rats + ezetimibe (ezetrol)

Table 4. Short chain fatty acids (SCFAs) concentration and pH of caecal digesta

Group	*SCFAs concentration (mMol/L)*			*pH*
	Acetate	*Propionate*	*Butyrate*	
NC	69.62 ± 14.36[d]	44.49 ± 9.47[d]	15.30 ± 7.33[c]	6.33 ± 0.04[a]
PC	13.44 ± 6.81[a]	7.20 ± 2.97[a]	2.02 ± 1.29[a]	6.81 ± 0.02[d]
WM	26.01 ± 9.65[ab]	14.98 ± 4.11[ab]	4.22 ± 1.09[ab]	6.41 ± 0.01[bc]
RM	62.19 ± 7.21cd	39.88 ± 4.26[cd]	11.95 ± 1.83c	6.36 ± 0.02[ab]
FS	49.32 ± 11.10[bcd]	31.53 ± 7.79[cd]	10.11 ± 3.06[c]	6.38 ± 0.02[ab]
ET	38.71 ± 4.25[abc]	24.01 ± 4.62[bc]	7.85 ± 1.89b[c]	6.47 ± 0.03[c]

Values (mean ± SD, n = 3) in the same column with different letters indicated significant difference, ANOVA followed by Tukey HSD ($P < 0.05$).
NC negative control, PC positive control, WM hypercholesterolemia rats + WM moringa leaves flour, RM hypercholesterolemia rats + RM moringa leaves flour, FS hypercholesterolemia rats + commercial plant stanol esters containing fermented dairy product, ET hypercholesterolemia rats + ezetimibe (ezetrol)

to WM, FS, and ET groups. Table 4 shows that pH of caecal digesta was significantly different among the six groups ($P < 0.05$). Compared to PC group, NC group and treated groups had lower pH value of caecal digesta.

Total cholesterol and low density lipoprotein (LDL) cholesterol levels

The changes in rat serum TC and LDL-c levels after 4 weeks of intervention period are shown in Table 5. As shown in Table 5, there was about 2.38% and 0.65% increase in final serum TC level in NC and PC groups, respectively, in comparison to initial serum TC level. Whereas, WM, RM, FS, and ET significantly decreased serum TC level by 42.00%, 48.83%, 48.38%, and 52.82%, respectively. There was a significant difference in serum TC level among the six groups. Serum TC levels in treated groups were significantly lower compared to the PC group. The results show that the RM group had significantly lower serum TC levels than the WM group, but was not significantly different from FS and ET groups.

Table 5. Serum total cholesterol and low density lipoprotein (LDL) cholesterol concentration

	NC	*PC*	*WM*	*RM*	*FS*	*ET*
Total cholesterol (mg/dL)[#]						
Initial	76.28±1.72*	217.09±4.67*	212.38±4.99*	206.69±4.19*	215.60±3.62*	210.77±8.39*
Final	78.10±1.52*[a]	218.50±4.76*[e]	127.43±6.00*[d]	105.77±3.42*[bc]	111.30±6.58*[c]	99.45±2.40*[b]
Change (%)	+2.38	+0.65	-42.00	-48.83	-48.38	-52.82
LDL cholesterol (mg/dL)[#]						
Initial	25.17±2.73*	65.43±3.93*	65.56±4.52*	64.37±1.90*	66.49±4.63*	66.73±3.02*
Final	25.77±2.63*[a]	68.90±4.10*[d]	45.69±3.20*[c]	39.15±1.59*[b]	41.28±3.80*[bc]	35.59±1.59*[b]
Change (%)	+2.38	+5.30	-30.31	-39.19	-37.91	-46.67

([#]) Values (mean ± SD, $n = 5$) in the same row with different letters indicate significant difference, ANOVA followed by Tukey HSD ($P < 0.05$).
([+]) = indicates the increased value, (-) = indicates the decreased value, * = between initial and final value are significantly different at the 5% level by independent samples *t*-test.
NC negative control, PC positive control, WM hypercholesterolemia rats + white cultivar Moringa leaves flour, RM hypercholesterolemia rats + red cultivar Moringa leaves flour, FS hypercholesterolemia rats + commercial plant stanol esters containing fermented dairy product, ET hypercholesterolemia rats ezetimibe (ezetrol)

In line with serum TC level reduction, Table 5 shows that serum LDL-c level of treated groups also experienced a significant reduction. There is a significant difference in serum LDL-c levels among the six groups. We found significantly lower serum LDL-c level in the RM group compared to WM group. However, serum LDL-c level of the WM group is not significantly different to FS group. Meanwhile, there were no statistical differences among serum LDL-c level in RM, FS, and ET groups.

DISCUSSION

This study showed that administration of WM and RM moringa leaves flour significantly decreased serum TC level (42.00 and 48.83%, respectively) in rats compared to untreated rats that had a 0.65% increase in TC levels (Table 5). Similar results were previously reported (Priyadarshani, Pratap & Varma, 2013; Bais, Singh & Sharma, 2014). The hypocholesterolemic effect of moringa leaves flour of both types in hypercholesterolemia rats may be explained by mechanisms involved in increasing faecal cholesterol and formation of SCFA in the digestive tract, as shown by higher faecal cholesterol in rats fed WM and RM moringa leaves flour, compared to untreated rats. The presence of SDF and phytosterols in the moringa leaves flour might have a role in reducing intestinal cholesterol absorption and enhancing cholesterol elimination into faeces. Dietary fibres are known to increase the viscosity of intestinal digesta and thus reduce cholesterol absorption. However, in our study we found no differences in the viscosity of small intestinal digesta among all groups. This was probably due to the presence of 5% carboxymethyl cellulose (CMC) as the fibre source in the AIN-93M standard diet. Hashemipour *et al.* (2014) reported that broilers fed with standard diet containing CMC had an increase in the viscosity of intestinal digesta.

The presence of phytosterols in both types of moringa leaves flour might have also contributed to increased faecal cholesterol excretion due to their ability to competitively inhibit the absorption of cholesterol. Phytosterols have a similar chemical structure to cholesterol and higher affinity to micelles and are able to displace cholesterol from mixed micelles (Pattel & Thompson, 2006), thus inhibiting the absorption of cholesterol, and subsequently increasing fecal cholesterol excretion. In the enterocyte, phytosterols remain in the free form due to their poor esterification by acyl coenzyme A/cholesterol acyltransferase (ACAT). Furthermore, those free phytosterols enhance efflux of cholesterol by inducing a higher Abcg5/g8 transporter expression (Plat & Mensink, 2005). Wang *et al*. (2015) found that hamsters fed with a high cholesterol diet containing β-sitosterol exhibited a significant decrease in cholesterol absorption. In line with the decrease in cholesterol absorption, the excretion of faecal total neutral sterols including cholesterol increased. Other studies also revealed that the administration of phytosterols increased neutral faecal sterol excretion and decreased hepatic cholesterol concentration (Plösch *et al*., 2006).

The WM group showed higher reduction levels of TC and LDL-c than the WM group. As the phytosterols content of RM (252 ppm) was lower than WM (723 ppm), presumably dietary fibre might have contributed to the reduction since RM moringa leaves flour had higher fibre content than WM leaves.

Ferreira *et al*. (2008), Razis, Ibrahim & Kntayya (2014), and Leone *et al*. (2015) reported that generally moringa leaves contain other bioactive compounds such as tocopherols, isoflavones, flavonoids, phenolics, and others, which might also contribute to lower cholesterol level.

Compared to WM group, the FS group had higher faecal cholesterol concentration

because it had more phytosterol derivatives (rats consumed plant stanol esters 71-80 mg/d) than WM moringa leaves flour (rats consumed phytosterols 0.12-0.13 mg/d). The FS group were fed with fermented dairy products that contained plant stanol esters 1.7 g/100 ml. Plant stanol esters are more soluble in various foods such as margarine as well as in the intestinal tract, thus interfering in the absorption of cholesterol. It was therefore more effective in increasing faecal cholesterol excretion (De Smet, Mensink & Plat, 2012). The presence of lactic acid bacteria in this product was also able to inhibit the absorption of cholesterol. It has been reported that *Lactobacillus*-fermented adlay-based milk enhances faecal cholesterol excretion (Wang *et al.,* 2010).

Compared to RM group, faecal cholesterol concentration of the FS group was significantly lower (Table 3) offering evidence that the ability of dietary fibre in inhibiting cholesterol absorption is higher than phytosterols. High excretion of faecal cholesterol in RM group was most likely caused by dietary fibre which was higher in RM moringa leaves flour compared to the white one (WM). Therefore, despite lower phytosterols content than WM moringa leaves flour (723 ppm) and plant stanol ester containing food (1.7 g/100 ml), RM moringa leaves flour (phytosterols of 252 ppm) produced greater faecal cholesterol excretion. Thus the reduction of TC and LDL-c in the group of rats treated by RM was higher than in the WM treated rats (Table 5).

Treatment with ezetimibe (ezetrol) was more effective in inhibiting cholesterol absorption (more faecal cholesterol excretion) than with RM and WM moringa leaves flour. Ezetimibe is a cholesterol lowering drug that acts as cholesterol absorption inhibitor by blocking Niemann-Pick C1-Like 1 (NPC1L1) protein in the intestinal epithelial cell brush border membrane (Bays *et al*., 2008). Ezetimibe also inhibits hepatic NPC1L1 (Temel *et al*., 2007), that is expected to increase biliary cholesterol saturation index thereby lowering blood cholesterol levels. On the other hand, the inhibition of hepatic NPC1L1 by ezetimibe potentially promotes formation of cholesterol gallstones (Jia, Betters & Yu, 2011). High ability in inhibiting cholesterol absorption implies that TC and LDL-c reduction was highest in the group of rats treated by ezetrol.

SCFAs as colonic fermentation products of soluble dietary fibre could inhibit cholesterol synthesis. Wong *et al*. (2006) reported that SCFAs, especially acetate and propionate, inhibited synthesis of cholesterol. Propionate suppresses cholesterol synthesis by inhibiting HMG-CoA reductase and increasing the amount of cholesterol that is converted into bile acids by increasing cholesterol 7α hydroxylase activity (Arora, Sharma & Frost, 2011). Meanwhile, acetate is able to reduce serum TC level by inhibiting hepatic lipogenesis and elevating faecal bile acid excretion (Fushimi *et al*., 2006). More SCFAs were formed in the caecal digesta of RM group than in the caecal digesta of WM group (Table 4) because of higher dietary fibres as the substrate of bacterial fermentation. Drzikova, Dongowski & Gebhardt (2005) revealed that supplementation of a diet with oat bran significantly enhanced SCFAs (acetate, propionate, and butyrate) concentration compared to control. Acidic pH of caecal digesta (Table 4) probably played a significant role in controlling bile acids reabsorption. Effectiveness of bile acids reabsorption in the colon depends on the solubility of bile acids in the caecum, which is reduced by acidic pH of caecal digesta (Moundras *et al*., 1997). As a consequent, it causes an increase in faecal bile acid excretion, thus reducing bile acids returned to the liver in the enterohepatic cycle.

In line with serum TC level reduction, administration of WM and RM moringa leaves flour also significantly lowered

serum LDL-c levels in rats compared to untreated rats (Table 5). This result was in accordance with previous studies (Priyadarshani *et al*., 2013; Bais *et al*., 2014), which found that moringa leaves flour and methanolic extract of moringa leaves not only lowered serum TC level but also lowered serum LDL-c level. Underlying mechanisms of this LDL-c lowering effect were probably a response to the inhibitory absorption of cholesterol and fat, elevation of bile secretion into the intestine, and elevation of faecal cholesterol and bile acids excretion. Dietary fibres and phytosterols in WM and RM moringa leaves flour were able to inhibit intestinal cholesterol and fat absorption which led to an increase in bile acid secretion into the intestinal lumen. This condition caused a reduction in hepatic bile acid levels, thus increasing hepatic degradation of cholesterol into bile acids (Lefebvre *et al*., 2009), implying a decreasing hepatic cholesterol level. A low level of hepatic cholesterol leads to a compensatory increase in hepatic LDL receptor activity thereby increasing the uptake of LDL-c, with a consequent decrease in serum LDL-c level.

Our data shows that the hypo-cholesterolemic effect of RM moringa leaves flour was similar to fermented dairy products that contain plant stanol esters. Meanwhile, the hypocholesterolemic effect of WM moringa leaves flour especially in reducing serum LDL-c was the lowest. The highest improvement of TC and LDL-c was found in the group of rats treated by ezetrol. Different mechanisms in choles-terol inhibition was found to result in different levels of reduction. However, ezetimibe also shows common adverse reactions such as headache and/or diarrhoea (steathorrea). Infrequent adverse effects are myalgia and/or raised liver function, and rarely hypersensitivity reactions (rash, angioedema) or myopathy (Patel *et al*., 2011). Therefore, moringa leaves flour can be an alternative for management of hypercholesterolemia.

CONCLUSION

This study demonstrates that RM moringa leaves flour are better than WM in reducing serum TC and LDL-c levels, increasing faecal cholesterol and SCFAs concentrations, and reducing pH value of caecal digesta due to its higher dietary fibre content. The reduction of TC and LDL-c is related to inhibition of cholesterol absorption thus increasing fecal cholesterol concentration. Moreover, it is also related to inhibition of cholesterol synthesis by SCFAs. This study found that WM and RM moringa leaves flour had hypocholesterolemic activity. The hypocholesterolemic activity of moringa leaves flour at tested dose (0.822 mg/g bw/d) was similar to commercial fermented dairy products containing plant stanol ester (tested dose 0.02 ml/g bw/d) and a drug for reducing cholesterol (ezetrol, tested dose 0.001 mg/g bw/d). Thereby, it could be considered as an ingredient for functional food to prevent hypercholesterolemia. Based on the results obtained in this study, the authors estimate about 8 g/day of moringa leaves should be consumed by humans to benefit from the hypocholesterolemic effect.

REFERENCES

AOAC (2005). Official Methods of Analysis of AOAC International, 18th ed. Gaithersburg MD USA.

Arora T, Sharma R & Frost G (2011). Propionate: Anti-obesity and satiety enhancing factor. *Appetite* 56(2): 511–515.

Asp NG, Johansson CG, Hallmer H & Siljestrom M (1983). Rapid enzimatic assay of insoluble and soluble dietary fibers. *J Agric Food Chem* 31(3): 476-482.

Bais S, Singh GS & Sharma R (2014). Antiobesity and hypolipidemic activity of *Moringa oleifera* leaves against high fat diet-induced obesity in rats. *Advances in Biology* Article ID 162914.

Bays HE, Neff D, Tomassini JE & Tershakovec AM (2008). Ezetimibe: cholesterol lowering

and beyond. *Expert Rev in Cardiovascular Therapy* 6(4): 447-470.

De Smet E, Mensink RP & Plat J (2012). Effects of plant sterols and stanols on intestinal cholesterol metabolism: Suggested mechanisms from past to present. *Mol Nutr Food Res* 56(7): 1058-1072.

Drzikova B, Dongowski G, & Gebhardt E (2005). Dietary fibre-rich oat-based products affect serum lipids, microbiota, formation of short-chain fatty acids and steroids in rats. *Br J Nutr* 94(6): 1012-1025.

Ferreira PMP, Farias DF, Oliveira JTDA & Carvalho ADFU (2008). *Moringa oleifera:* bioactive compounds and nutritional potential. *Rev Nutr Campinas* 21(4): 431-437.

Fushimi T, Suruga K, Oshima Y, Fukiharu M, Tsukamoto Y & Goda T (2006). Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet. *Br J Nutr* 95(5): 916-924.

Hashemipou H, Kermanshahi H, Golian A & Khaksar V (2014). Effects of carboxy methyl cellulose and thymol + carvacrol on performance, digesta viscosity and some blood metabolites of broilers. *J Anim Physiol Anim Nutr* 98(4): 672-679.

Jia L, Betters JL & Yu L (2011). Niemann-Pick C1-Like 1 (NPC1L1) protein in intestinal and hepatic cholesterol transport. *Annu Rev Physiol* 73: 239-259.

Kiranawati TM, Harijono, Estiasih T & Sriwahyuni E (2012). Nutrient content of kelor (*Moringa oleifera* Lamk) leaves powder under different blanching methods. *Food Public Health* 2(6): 296-300.

Kristensen M, Jensen MG, Aarestrup J, Petersen KEN, Søndergaard L, Mikkelsen MS & Astrup A (2012). Flaxseed dietary fibers lower cholesterol and increase fecal fat excretion, but magnitude of effect depends on food type. *Nutr Metab* 9(8): 1-8.

Lefebvre P, Cariou B, Lien F, Kuipers F & Staels B (2009). Role of bile acids and bile acid receptors in metabolic regulation. *Physiol Rev* 89(1): 147-191.

Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J & Bertoli S (2015). Cultivation, genetic, ethnopharmacology, phyto-chemistry and pharmacology of *Moringa oleifera* leaves: an overview. *Int J Mol Sci* 16: 12791-12835.

Moreau RA, Whitaker BD & Hicks KB (2002). Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Prog Lipid Res* 41: 457-500.

Moundras C, Behr SR, Rémésy C & Demigné C (1997). Fecal losses of sterols and bile acids induced by feeding rats guar gum are due to greater pool size and liver bile acid secretion. *J Nutr* 127(6): 1068-1076.

Otunola GA, Oloyede OB, Oladiji AT & Afolayan AA (2010). Effects of diet-induced hypercholesterolemia on the lipid profile and some enzyme activities in female Wistar rats. *Afr J Biochem Res* 4(6): 149-154.

Passos AA, Park I, Ferket P, von Heimendahl E & Kim SW (2015). Effect of dietary supplementation of xylanase on apparent ileal digestibility of nutrients, viscosity of digesta, and intestinal morphology of growing pigs fed corn and soybean meal based diet. *Anim Nutr* 1(1): 19-23.

Patel MD & Thompson PD (2006). Phytosterols and vascular disease. *Atherosclerosis* 186(1): 12-19.

Patel KM, Shah DH, Patel JB, Patel JS, Garg CS & Sen DJ (2011). Chemistry of four membered heterocylic ezetimibe as lipid lowering agent. *Int J Drug Dev & Res* 3(2): 104-110.

Plat J & Mensink RP (2005). Plant stanol and sterol esters in the control of blood cholesterol levels: mechanism and safety aspects. *Am J Cardiol* 96(1a): 15d-22d.

Plösch T, Kruit JK, Bloks VW, Huijkman NCA, Havinga R, Duchateau GSMJE, Lin Y & Kuipers F (2006). Reduction of cholesterol absorption by dietary plant sterols and stanols in mice is independent of the Abcg5/8 transporter. *J Nutr* 136(8): 2135-2140.

Plummer DT (1977). An Introduction to Practical Biochemistry. McGraw-Hill Companies, New York.

Priyadarshani N, Pratap R & Varma MC (2013). Altered lipid profile of diabetic mice and

hypolipidemic role of *Moringa oleifera* Lam. leaf powder. *Int J Appl Biosci* 1(3): 28-34.

Rajanandh MG, Satishkumar MN, Elango K & Suresh B (2012). *Moringa oleifera* Lam. a herbal medicine for hyperlipidemia: a preclinical report. *Asian Pac J Trop Dis* 2012: S790-S795.

Razis AFA, Ibrahim MD & Kntayya SB (2014). Health benefits of *Moringa oleifera. Asian Pac J Cancer Prev* 15(20): 8571-8576.

Slavin M & Yu L (2012). A single extraction and HPLC procedure for simultaneous analysis of phytosterols, tocopherols and lutein in soybeans. *Food Chem* 135(4): 2789-2795.

Temel RE, Tang W, Ma Y, Rudel LL, Willingham MC, Ioannou YA, Davies JP, Nilsson LM & Yu L (2007). Hepatic Niemann-Pick C1-like 1 regulates biliary cholesterol concentration and is a target of ezetimibe. *J Clin Invest* 117(7): 1968-1978.

Wang CY, Wu SC, Ng CC, & Shyu YT (2010). Effect of *Lactobacillus*-fermented adlay-based milk on lipid metabolism of hamsters fed cholesterol-enriched diet. *Food Res Int* 43(3): 819-824.

Wang X, Huang W, Lei L, Liu Y, Ma KY, Li YM, Wang L, Huang Y & Chen ZY (2015). Blockage of hydroxyl group partially abolishes the cholesterol-lowering activity of β-sitosterol. *J Funct Foods* 12: 199-207.

Weber N, Weitkamp P & Mukherjee KD (2002). Cholesterol-lowering food additives: lipase-catalysed preparation of phytosterol and phytostanol esters. *Food Res Int* 35(2–3): 177-181.

Wong JMW, de Souza R, Kendall CWC, Emam A & Jenkins DJA (2006). Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* 40(3): 235-243.

World Health Organization (2015). Global Status Report on Noncommunicable *Diseases 2014. From* http://www.who.int [Retrieved September 15 2015].

Zhou H & Hylemon PB (2014). Bile acids are nutrient signaling hormones. *Steroids* 86: 62-68.