The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves

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Abstract

Moringa oleifera is used in Thai traditional medicine as cardiotonic. Recent studies demonstrated its hypocholesterolaemic effect. However, to be clinically useful, more scientific data are needed. *Aim of the Study*: We investigated the antioxidant, hypolipidaemic and antiatherosclerotic activities of *Moringa oleifera* leaf extract. *Materials and Methods*: Scavenging activity of the extract on 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH), and the inhibitory effect on Cu^{2+}-induced low-density lipoprotein (LDL) oxidation were determined in *in vitro* experiment. The effects of the extract on cholesterol levels, conjugated diene (CD) and thiobarbituric acid reactive substances (TBARS) and plaque formations in cholesterol-fed rabbits were investigated.

Results: We found that in scavenging DPPH radicals the extract and Trolox® had IC_{50} of 78.15 ± 0.92 and 2.14 ± 0.12 μg/ml, respectively. The extract significantly ($P < 0.05$) prolonged the lag-time of CD formation and inhibited TBARS formation in both *in vitro* and *ex vivo* experiments in a dose-dependent manner. In hypercholesterol-fed rabbits, at 12 weeks of treatment, it significantly ($P < 0.05$) lowered the cholesterol levels and reduced the atherosclerotic plaque formation to about 50 and 86%, respectively. These effects were at degrees comparable to those of simvastatin.

Conclusions: The results indicate that this plant possesses antioxidant, hypolipidaemic and antiatherosclerotic activities and has therapeutic potential for the prevention of cardiovascular diseases.
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Keywords: Moringa oleifera Lam. leaves; Moringaceae; Hypolipidaemic; Antiatherosclerotic

1. Introduction

Atherosclerosis is a disease of blood vessels and known colloquially as "hardening of the arteries". It is characterized by the accumulation of fatty substance, cholesterol, cellular waste products, calcium and other substances in the inner lining of an artery. Major complications of atherosclerosis include angina pectoris, myocardial infarction and stroke, which are recognized as leading causes of morbidity and mortality in Western countries. The World Health Organization (WHO) predicted that heart diseases and stroke are becoming more deadly, with a projected combined death toll of 24 million by 2030 (Reinhardt, 2005).

Atherosclerosis involves many processes such as hypercholesterolaemia, oxidation and inflammation (Lowenstein and Matsushita, 2004). Oxidized low-density lipoprotein (LDL) and endothelial dysfunction have been found to play a pivotal role in the pathogenesis of atherosclerosis (Lusis, 2000). It has been well established that antioxidants significantly arrest athero-

genesis in rabbits, hamsters, mice and non-human primates (Chisolm and Steinberg, 2000). Many herbal medicines and foodstuff are believed to have preventive effects on chronic diseases due to their radical scavenging or antioxidant properties (Potterat, 1997). In herbal products, phenolic compounds have been shown to be effective antioxidant constituents. Many polyphenolics exert more powerful antioxidant effect than vitamin E *in vitro* and inhibit lipid peroxidation by chain-breaking peroxyl-radical scavenging. They can also directly scavenge reactive oxygen species (ROS), such as hydroxyl, superoxide and peroxynitrite radicals (Tsao and Akhtar, 2005).

Moringa oleifera Lam. (drumstick tree, horse-radish tree, synonym: *Moringa pterygosperma* Gaertn.), a member of the family Moringaceae, is a small-medium sized tree, 10–15 m high, widely cultivated in East and Southeast Asia, Polynesia and the West Indies. Different parts of the *Moringa oleifera* tree are reported to possess various pharmacological actions. The leaves and fruits are found to have hypocholesterolaemic activity in Wistar rats and rabbits, respectively (Ghasi et al., 2000; Mehta et al., 2003). The leaves as well as flowers, roots, gums and fruits are extensively used for treating inflammation (Ezeamuzle et al., 1996) and cardiovascular diseases (Limaye et al., 1995). In addition, the leaves can serve as a rich source of beta-carotene (Nambiar and Seshadri, 2001), vitamin C and E, and polyphenolics (Ross, 1999). In Thailand, the tender pods, fruits and leaves of *Moringa oleifera* have been consumed as vegetables for more than 100 years, while hot water extract of the dried roots was taken orally as a cardiotonic, a stimulant against fainting (Mokkhasmit et al., 1971). The potential therapeutic values against cancer, diabetes, rheumatoid arthritis and other diseases have earned this plant the name of "wonder tree" in Thailand. Since the life style of Thais have become more westernized, there have been increases in cases of cardiovascular diseases, which are the fourth leading causes of death in 2003 and are becoming major health and economic problems for Thailand (Rukumnuaykit, 2004). Searching for herbs that have therapeutic potential for the prevention and scientifically proven to be useful as an alternative treatment is needed. If those herbs were table vegetable and widely consumed, the advantage would be enormous. Thus, the aims of the present study are to determine the *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities and the phenolic content of water extract of *Moringa oleifera* leaves. This plant is indigenous to many Asian countries, and is abundant and cheap food source. Thus, any health benefit from this plant is likely to reach a large part of the population.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicals, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), 1,1,3,3-tetraethoxy-propane (TEP), (+)-α-tocopherol (vitamin E), albumin (bovine), butylated hydroxytoluene (BHT), Folin-Ciocalteu's phenol reagent, potassium bromide, sodium phosphate monobasic anhydrous, thiobarbituric

acid, trichloroacetic acid, gallic acid, and sodium carbonate were purchased from Sigma (St. Louis, MO, USA). Absolute ethanol, copper sulfate pentahydrate, disodium hydrogen phosphate, methanol, potassium sodium tartrate tetrahydrate, sodium carbonate, sodium chloride, sodium dihydrogen phosphate anhydrous, and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Ketamine (Calyosol®) was purchased from Gedeon-Richter (Hungary). Pentobarbital (Nembutal®) was purchased from Abbott (North Chicago, IL. USA). Other unstated chemicals and reagents were of analytical grade.

2.2. Plant material and water extraction

Moringa oleifera leaves were collected from Trang province, Thailand. Voucher herbarium specimen has been deposited in the Forestry Herbarium, Royal Forest Department, Bangkok (BKF-PC-1). Two hundred grams of fresh leaves were blended with 1 l of sterile distilled water and boiled for 5 min and left to cool down to room temperature. The decoction solution was filtered through filter paper using a suction apparatus, and the filtrates were pooled and lyophilized. The dried extract was ground to powder, which was kept in a freezer at −40 °C. The yield (w/w) of freeze-dried powder from fresh leaves was about 10%.

2.3. Scavenging activity on DPPH radicals

The scavenging activity of the *Moringa oleifera* leaf extract on DPPH radicals was assayed according to the method described previously (Roche et al., 2005). Thirty microliters of aqueous solution containing various concentrations of the leaf extract powder (50–400 μg/ml) were mixed with 3 ml of 0.2 mM DPPH in methanol. Absorbance at 515 nm was determined every 30 s for 6 min after adding the extract. Trolox® (1–5 μg/ml) was used as the standard antioxidant. The DPPH radical scavenging activity of the test substance was calculated by the following equation: scavenging activity (%) = 1 − (absorbance at 515 nm of 0.2 mM DPPH plus test substance)/(absorbance at 515 nm of 0.2 mM DPPH) × 100.

From this experiment, the 50% DPPH radical scavenging concentrations (IC_{50}) of the test substances were calculated.

2.4. Inhibitory effect on Cu^{2+}-induced LDL oxidation

Human plasma was obtained from EDTA-treated blood and separated by low-speed centrifugation at 2330 g at 4 °C for 10 min. The plasma sample was stored at −20 °C and used within 1 week for LDL isolation. LDL with a density of 1.019–1.063 g/ml was isolated by a sequential ultracentrifugation method (Redgrave et al., 1975) and dialyzed against 10 mM phosphate buffered saline (PBS) pH 7.4, at 4 °C for 24 h. Protein concentration in the LDL sample was determined by the modified method of Lowry (Markwell et al., 1978).

To measure the effect of leaf extract powder on conjugated diene (CD) formation, LDL, adjusted to 35 μg protein/ml with 10 mM PBS pH 7.4 was preincubated at 37 °C for 1 h with various concentrations of freeze-dried *Moringa oleifera* leaf extract

(1, 10, 30 and 50 μg/ml). Vitamin E, as a standard antioxidant, was dissolved in absolute ethanol, dried under nitrogen and incubated with LDL (final vitamin E concentration was 100 μM). Oxidation reaction of LDL was initiated by adding freshly prepared 10 μM CuSO$_4$ solution and incubated at 37 °C for 6 h. CD formation during oxidation of LDL was continuously monitored by a spectrophotometric method based on the changes in absorbance at 234 nm using a double beam spectrophotometer (Cintra 10e GBC).

To determine the formation of thiobarbituric acid reactive substances (TBARS), human LDL was preincubated with different concentrations of the leaf extract powder or vitamin E as described above. After initiating the oxidation process with CuSO$_4$, an aliquot of the reaction mixture was removed every hour and the reaction was terminated with the addition of 50 μl of 100 mM butylated hydroxytoluene (BHT). Afterwards, 1 ml of 10% trichloroacetic acid (TCA), 0.5 ml of 5 mM disodium ethylenediamine (Na$_2$EDTA), 1.5 ml of 8% sodium dodecyl sulfate (SDS) and 1.5 ml of 0.6% thiobarbituric acid (TBA) were added to each 1 ml aliquot of the reaction. After incubation at 80 °C for 1 h, TBARS formation was measured by using a spectrofluorometer (PerkinElmer SL55) at an excitation wavelength of 515 nm and an emission wavelength of 553 nm. 1,1,3,3-tetraethoxypropane (TEP) was used as standard.

2.5. Determination of total phenolic content

Total phenolic content of the leaf extract powder was determined with Folin-Ciocalteu's reagent modified from the method of Waterman and Mole (1994) using gallic acid as a standard. One hundred milligrams of the freeze-dried powder was extracted with 10 ml of 40% ethanol and sonicated for 30 min, vortexed for 10 min and allowed to stand at room temperature for 1 h. The extract was filtered through a Whatman no.1 filter (Whatman Inc., Clifton, NJ). At least 4 ml of the filtrate was collected. One hundred microliters of the filtrate was transferred to a 10 ml volumetric flask and 6 ml of water was added. The contents were swirled to mix; 0.5 ml of Folin-Ciocalteu's phenol reagent was added and mixed again. After 1 min and before 8 min, 1.5 ml of 20% sodium carbonate solution was added and the time was recorded as time zero, and the contents were mixed again. The volume was made up to 10 ml with water and mixed thoroughly. The solution was left for 2 h and the absorption at 760 nm was recorded. Various concentrations of gallic acid (25, 50, 100, 200, and 400 μg/ml) were used as the standards.

2.6. Experiments in animals

Animal use protocol was approved by the Srinakarinwirot University Animal Use Committee and was in accordance with International Standard on the care and use of experimental animals (CCAC, 1993).

Thirty-two healthy male adult New Zealand white rabbits, weighing 1.5–2.0 kg, were housed individually in large cages with free access to food and water *ad libitum* during the course of the experiment. These animals were held at a 12/12 h light/dark cycle, 18–22 °C, and relative humidity of 40–60%. After a 4-week period of acclimatization, the rabbits were randomly divided into four groups of eight animals each, and reared under experimental conditions for 12 weeks. Group I (Control group) rabbits were fed with standard laboratory diet (SLD); group II (HCD group) rabbits were fed with high cholesterol diet (HCD) containing 0.5% cholesterol and 99.5% SLD; group III (Simvastatin group) rabbits were fed with HCD plus simvastatin (5 mg/kg/day, p.o.); group IV (*Moringa oleifera* group) rabbits were fed with HCD plus freeze-dried powder of *Moringa oleifera* leaf extract (0.1 g/kg/day, p.o.).

Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) levels in the plasma of rabbits were determined at the beginning of the experimental period and at 4 weeks thereafter. Blood samples were drawn by puncture of the central ear vein into vacutainers containing 1.2 mg EDTA/ml whole blood. Plasma was separated by centrifuging blood sample at 2330 × g at 4 °C for 10 min. The plasma was kept at -70 °C until analysis.

At the end of 12-week experiment, rabbits were premedicated with ketamine (25 mg/kg body weight, i.m) followed by general anaesthesia with sodium pentobarbital via the marginal ear vein (initial dose 40 mg plus maintenance dose as required). After blood samples were taken, the animals were sacrificed with overdose of pentobarbital. The carotid arteries were removed, dissected free of adherent fat and fascia, and kept at -20 °C. The isolation of rabbit LDL, Cu^{2+}-induced rabbit LDL oxidation, CD and TBARS formations were determined as described above.

Each carotid artery was sliced into stripes of 2 cm in length and plaque formation on the internal surface was photographed by using a Sony digital still camera (DSC-707). The level of plaque formation on the internal carotid arteries was calculated by using the equation: (area of plaque formation on internal carotid arteries/total area) × 100.

2.7. Statistical analysis

All data were expressed as mean ± S.E. of the mean (S.E.M.). Data from *in vitro* and *ex vivo* experiments were obtained from three independent experiments, each performed with duplicate measurements. For statistical analysis, Student's *t*-test for unpaired data was used. Comparison among experimental animal was performed using multiple-comparison analyses of variance (ANOVA) followed by Scheffe's test. Differences between the data were considered significant at $P < 0.05$.

3. Results

3.1. Scavenging effects on DPPH radicals

To determine whether the *Moringa oleifera* leaf extract had radical scavenging activities, we measured its effect in scavenging DPPH radicals. The leaf extract had an IC$_{50}$ of 78.15 ± 0.92 μg/ml. In comparison, the positive control, Trolox® had an IC$_{50}$ of 2.14 ± 0.12 μg/ml.

3.2. Effects on CD formation in human LDL

We measured the dynamics of CD formation in human LDL in the absence or presence of leaf extract. Incubation of human LDL 35 μg protein/ml with 10 μM $CuSO_4$-induced CD formation with a lag-time (the duration between time 0 to time when CD formation started) of 30.66 ± 1.76 min. In the presence of the leaf extract at a final concentration of 1, 10, 30, and 50 μg/ml, the lag-time of CD formation was increased to 45.0 ± 1.15, 151.66 ± 4.41, 251.67 ± 1.67, and 340.33 ± 1.66 min, respectively. In comparison, 100 μM vitamin E delayed the lag-time to 100 ± 0.05 min, which was equivalent to about 5 μg/ml of the leaf extract. These results demonstrated that the *Moringa oleifera* leaf extract significantly delayed the lag-time of CD formation in a dose-dependent manner and the IC_{50} at 2 h of the reaction was 5 μg/ml.

3.3. Effects on TBARS formation in human LDL

The effect of the leaf extract on the formation of TBARS resulting from the oxidation of human LDL was shown in Fig. 1. Incubation of 35 μg protein/ml of human LDL with 10 μM $CuSO_4$ at 37 °C for 6-h induced TBARS formation (Fig. 1A). At 2 h of the incubation period, (Fig. 1B) TBARS formation of the reaction mixture was 120.67 ± 6.20 nmol/mg LDL protein. In the presence of *Moringa oleifera* leaf extract at a final concentration of 1 and 10 μg/ml, the TBARS formation was reduced to 104.16 ± 12.74 and 2.80 ± 1.40 nmol/mg LDL protein, respectively. Incubation with 30 or 50 μg/ml of the leaf extract completely blocked TBARS formation. In contrast, the presence of 100 μM vitamin E could only reduce TBARS formation to 70.14 ± 2.21 nmol/mg LDL protein. It was shown that vitamin E and the leaf extract at a concentration of 10 μg/ml significantly reduced TBARS formation ($P < 0.05$), while the leaf extract at the concentrations of 30 or 50 μg/ml completely blocked the TBARS formation.

3.4. Total phenolic content of the leaf extract of Moringa oleifera

The total phenolic content of the leaf extract powder was determined. It was found that 10 mg/ml of the extract was equivalent to 205.8 ± 0.22 μg/ml of gallic acid.

3.5. Experiments in rabbits

3.5.1. Body weight and plasma lipid concentrations

The body weight, levels of plasma cholesterol and triglycerides in 4 groups of rabbits during the 12 weeks of experiment were shown in Table 1. At the beginning of the experiment, there was no significant difference in body weight among the four groups of animals. The body weight of rabbits was continuously increased throughout the experimental period, but it was not significantly different ($P > 0.05$) among the four groups at the same time point of treatment. Plasma TC concentrations of HCD group (baseline 40.00 ± 6.16 mg/dl) was increased to
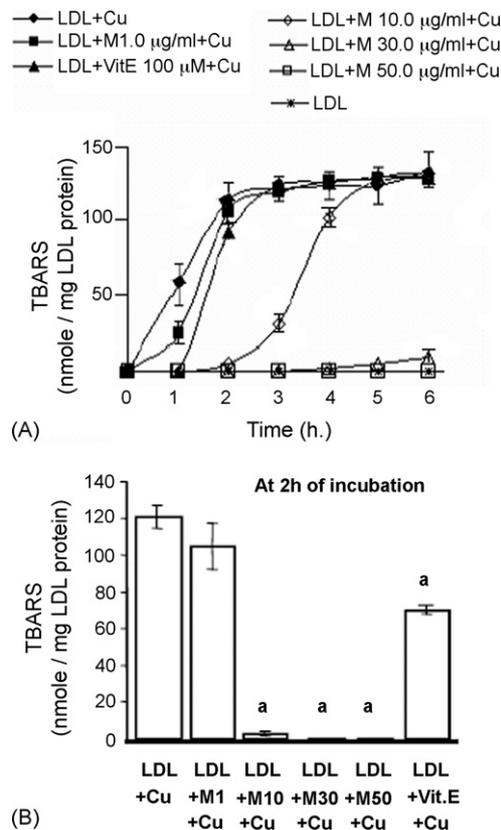

Fig. 1. The effects of *Moringa oleifera* leaf extract and vitamin E on the formation of TBARS in $CuSO_4$-induced human LDL oxidation. (A) TBARS formation (nmol/mg LDL protein) was determined every 1 h for 6 h after preincubation for 1 h with 1, 10, 30 and 50 μg/ml of the extract or 100 μM of vitamin E. (B) TBARS formation (nmol/mg LDL protein) at 2 h of incubation periods after adding 10 μM $CuSO_4$ into human LDL. 100 (M of vitamin E and the leaf extract at concentration of 10 μg/ml significantly reduced TBARS formation. TBARS formation was completely blocked by the extract at concentrations of 30 and 50 μg/ml. Values are mean ± S.E.M. from three independent experiments, each experiment was performed in duplicate. 'a' denotes $P < 0.05$, statistically significant difference from LDL + Cu. Cu denotes 10 μM $CuSO_4$.

1134.50 ± 147.65 mg/dl after 4 weeks of 0.5% cholesterol diet and to 1606.50 ± 96.51 mg/dl at the end of the experiment. At the same time points, simvastatin significantly lowered the levels of TC to 62.48, 63.79 and 67.22% of those of the HCD group ($P < 0.05$). Similarly, a significant reduction in TC levels was also evident in *Moringa oleifera* group, with the TC levels being reduced to 37.10, 48.43 and 52.00%, of those of the HCD group ($P < 0.05$). Changes in LDL-C and HDL-C levels paralleled plasma cholesterol in all treatment groups; and significant reduction in LDL-C and HDL-C was found in simvastatin and *Moringa oleifera* groups ($P < 0.05$). Interestingly, the reduction of plasma HDL-C and LDL-C levels in *Moringa oleifera* group was highly comparable to that in the simvastatin group ($P > 0.05$). In addition, both *Moringa oleifera* and simvastatin treatments significantly reduced TG levels as compared to the HCD group ($P < 0.05$). Plasma TG levels in simvastatin and *Moringa oleifera* group were not significantly different from those in the control group at the same time point of treatment ($P > 0.05$).

Table 1
Body weight (kg), plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations (mg/dl) in control, HCD, simvastatin, and *Moringa oleifera* groups of rabbits were shown

Parameter studied	Time of treatment (weeks)			
	Start ($T=0$)	4	8	12
Bodyweight (kg)				
Control group	2.10 ± 0.04	2.54 ± 0.11	2.81 ± 0.06	3.11 ± 0.17
HCD group	2.21 ± 0.09	2.63 ± 0.21	3.07 ± 0.28	3.23 ± 0.21
Simvastatin group	2.16 ± 0.14	2.43 ± 0.16	2.69 ± 0.11	2.86 ± 0.04
Moringa oleifera group	2.11 ± 0.09	2.48 ± 0.24	2.86 ± 0.13	2.99 ± 0.08
Total cholesterol (mg/dl)				
Control group	34.54 ± 2.64	31.26 ± 3.99	29.23 ± 3.12	28.83 ± 4.83
HCD group	40.00 ± 6.16	1134.50 ± 147.65[a]	1512.75 ± 23.72[a]	1606.50 ± 96.5[a]
Simvastatin group	41.75 ± 4.31	425.60 ± 169.96[a,b]	547.80 ± 233.64[a,b]	526.75 ± 203.81[a,b]
Moringa oleifera group	42.00 ± 4.62	714.67 ± 227.64[a,b]	780.17 ± 204.12[a,b]	771.33 ± 193.08[a,b]
LDL-C (mg/dl)				
Control group	13.67 ± 1.45	11.00 ± 2.25	10.83 ± 1.96	9.83 ± 2.18
HCD group	14.20 ± 1.93	1071.75 ± 155.66[a]	1306.75 ± 10.75[a]	1305.50 ± 87.19[a]
Simvastatin group	11.75 ± 1.44	492.00 ± 192.14[a,b]	643.75 ± 238.13[a,b]	453.50 ± 174.91[a,b]
Moringa oleifera group	25.50 ± 8.53	671.67 ± 186.86[a,b]	803.33 ± 212.44[a,b]	748.50 ± 196.23[a,b]
HDL-C (mg/dl)				
Control group	24.50 ± 1.23	25.61 ± 1.62	21.84 ± 1.71	19.74 ± 3.33
HCD group	30.80 ± 3.47	265.75 ± 20.23[a]	381.25 ± 28.45[a]	331.50 ± 42.51[a]
Simvastatin group	29.00 ± 3.03	123.40 ± 44.7[a,b]	163.40 ± 64.41[a,b]	145.75 ± 51.86[a,b]
Moringa oleifera group	30.17 ± 3.55	174.00 ± 42.69[a,b]	200.00 ± 47.08[a,b]	185.17 ± 41.12[a,b]
Triglyceride (mg/dl)				
Control group	73.33 ± 6.69	74.00 ± 8.48	83.50 ± 8.77	82.17 ± 6.39
HCD group	87.75 ± 5.41	237.25 ± 58.19[a]	210.00 ± 62.74[a]	320.00 ± 89.74[a]
Simvastatin group	78.80 ± 8.92	91.20 ± 26.84[b]	80.20 ± 17.39[b]	89.00 ± 32.13[b]
Moringa oleifera group	70.83 ± 8.76	85.33 ± 25.68[b]	78.83 ± 15.56[b]	78.67 ± 9.29[b]

Simvastatin and *Moringa oleifera* leaf extract significantly lowered the levels of TC, LDL-C, HDL-C and TG ($P < 0.05$). Values are mean $\pm$ S.E.M. from eight animals. Control group: rabbits fed with standard laboratory diet (SLD) for 12 weeks. HCD group: rabbits fed with high-cholesterol diet for 12 weeks. Simvastatin group: rabbits fed with HCD plus simvastatin (5 mg/day, p.o.) for 12 weeks. *Moringa oleifera* group: rabbits fed with HCD plus *Moringa oleifera* leaf extract (0.1 g/kg/day, p.o.) for 12 weeks.

[a] $P < 0.05$, statistically significant difference from control group at the same time point of treatment.
[b] $P < 0.05$, statistically significant difference from HCD group at the same time point of treatment.

Table 2
The lag-time (min) of conjugated diene (CD) formation and levels of TBARS (nmol/mg LDL protein) formation in four groups of rabbits were shown

Groups of animal	Lag-time of CD formation (min)	TBARS levels (nmol/mg LDL protein)
Control group	173.37 ± 23	0.99 ± 0.69
HCD group	106.66 ± 3.33[a]	14.41 ± 5.85[a]
Simvastatin group	140 ± 15.27[b]	2.31 ± 1.0[b]
Moringa oleifera group	162.5 ± 6.29[b]	2.46 ± 0.81[b]

The lag-time of CD formation was significantly reduced in HCD group when compared to control, simvastatin and *Moringa oleifera* groups ($P < 0.05$). Levels of TBARS formation (nmol/mg LDL protein) at 2 h after the incubation of 35 µg protein/ml of LDL with 10 µM CuSO$_4$ in HCD group was significantly higher than those of the control, simvastatin and *Moringa oleifera* groups ($P < 0.05$). Values are mean $\pm$ S.E.M. from eight animals per group. HCD denotes high cholesterol diet.

[a] $P < 0.05$, statistically significant difference from control group.
[b] $P < 0.05$, statistically significant difference from HCD group.

3.5.2. CD and TBARS formation of rabbit LDL

At the end of the experimental period, LDL from the plasma of all rabbits was isolated and the effect of leaf extract on CD and TBARS formations was determined. The lag-time of CD formation in the rabbit LDL of control, HCD, simvastatin and *Moringa oleifera* groups were 173.37 ± 23, 106.66 ± 3.33, 140 ± 15.27 and 162.50 ± 6.29 min, respectively. The lag-time of CD formation in the rabbit LDL of HCD group was significantly less than that in control group ($P < 0.05$), whereas the lag-time of CD for-

mation in rabbit LDL of simvastatin and *Moringa oleifera* group was not significantly different from the control group (Table 2).

The TBARS formations at 2 h of the incubation period after adding CuSO$_4$ in the rabbit LDL of HCD group was 14.41 ± 5.85 nmol/mg LDL protein whereas the levels in control, simvastatin, and *Moringa oleifera* groups were 0.99 ± 0.69, 2.31 ± 1.00 and 2.46 ± 0.81 nmol/mg LDL protein, respectively (Table 2). These data showed that simvastatin and *Moringa oleifera* leaf extract significantly inhibited TBARS formation
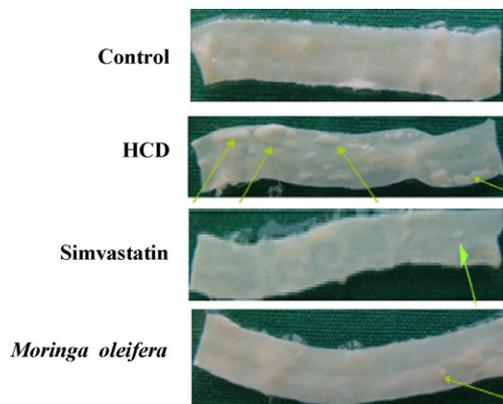
Fig. 2. The inhibitory effects of the *Moringa oleifera* leaf extract and simvastatin on the plaque formation in internal carotid arteries of rabbits fed with high cholesterol diet for 12 weeks. The figure represents the photographs of the internal surface of carotid arteries in four groups of rabbits. More plaque areas are shown in HCD than in simvastatin and *Moringa oleifera* as indicated by arrows. Control: control group; HCD: high cholesterol diet group; simvastatin: simvastatin group; *Moringa oleifera*: *Moringa oleifera* group.

in $CuSO_4$-induced rabbit LDL oxidation as compared to HCD group, ($P < 0.05$).

3.5.3. Aortic plaque formation

The internal surfaces of carotid arteries in the four groups of rabbits after 12 weeks of treatment were shown in Fig. 2. After 12 weeks of dietary intervention, a difference in atherosclerotic plaque in control, HCD, simvastatin and *Moringa oleifera* groups was evident at the simple inspection, there was no plaque formation on the internal carotid arteries in control animals, the percent of plaque formations in the HCD group was 20.4 ± 4.39. Whereas, the percent of plaque formations in simvastatin and in *Moringa oleifera* groups was 2.71 ± 1.98 and 2.75 ± 2.26, respectively. Simvastatin and *Moringa oleifera* leaf extract reduced the internal carotid atherosclerotic plaque formation to 86.72 and 86.52%, respectively, as compared to HCD group.

4. Discussion

Male New Zealand white rabbits fed with high cholesterol diet were used in the present study. Among experimental animal species, rabbits are well known for their tendency to develop severe hypercholesterolaemia when dietary cholesterol is increased (Ross et al., 1978) and to produce a strong relationship between the levels of plasma cholesterol and the degrees of aortic lesion with a marked thickening of the intima (Bocan et al., 1993). The distribution of atherosclerotic lesions in the rabbit model resembles atherosclerosis in humans to a certain degree (Wissler, 1992).

In this study, we show that diet containing 0.5% cholesterol significantly increased the levels of plasma TC, HDL-C, LDL-C and TG in rabbits, consistent with what have been reported previously (Prasad, 2005; Mehta et al., 2003). In addition, the results of our study clearly demonstrate for the first time that water extract of *Moringa oleifera* leaves significantly reduced the for-

mation of atherosclerotic plaque, along with levels of cholesterol and triglycerides. These results agree well with earlier findings where the water extract of the leaves of this plant significantly lowers the serum TC levels in hypercholesterolaemic Wistar rats (Ghasi et al., 2000), and the fruits of this plant significantly lowers the levels of serum cholesterol, very low-density lipoprotein and LDL in hypercholesterolaemic rabbits (Mehta et al., 2003). Simvastatin has been known to exert its lipid lowering effect by competitive inhibition of the hepatic HMG-CoA reductase. Importantly, it was demonstrated in this study that the *Moringa oleifera* leaf extract could reduce plaque formation, cholesterol and triglyceride levels in rabbits at degrees comparable to those of simvastatin.

We have shown that the *Moringa oleifera* leaf extract possessed strong radical scavenging activity and antioxidant activity. Polyphenols other than vitamin E have been known to exert powerful antioxidant effect *in vitro*. They inhibit lipid peroxidation by acting as chain-breaking peroxyl-radical scavengers, and can protect LDL from oxidation (Ò Byme et al., 2002). It has been found in the present study that the *Moringa oleifera* leaf extract contains 2% (w/w) of polyphenols, therefore, the antioxidant effects of the leaf extract may depend on its phenolic components.

Polyphenolic compounds also possess a variety of other biological activities, such as reduction of plasma lipids, which might be due to the up-regulation of LDL receptor expression (Kuhn et al., 2004), inhibition of hepatic lipid synthesis (Theriault et al., 2000) and lipoprotein secretion (Borradaile et al., 2003), and increase in cholesterol elimination via bile acids (Del Bas et al., 2005). It is possible that the activity in lowering lipid levels and aortic plaque formation of the *Moringa oleifera* leaf extract may result from the phenolic compounds present in the extract. However, the precise mechanisms underlying these effects need to be elucidated in future studies.

In the present study, the effects of *Moringa oleifera* leaf extract and vitamin E on oxidative modification of LDL by determining levels of TBARS and lag-time of CD formations were demonstrated. It has been found in this study that after the incubation of human LDL with copper, the production of CD and TBARS was increased. In contrast, in the presence of *Moringa oleifera* leaf extract or vitamin E, the oxidative modifications of LDL were significantly inhibited in a dose-dependent manner that was evident by the significant prolongation of the lag-time of CD formation and reduction in levels of TBARS formation. These findings indicate that *Moringa oleifera* leaf extract suppresses the initiation and propagation of lipid peroxidation, and owing to its phenolic content, it may help suppress atherosclerosis by scavenging hydrogen oxide radicals. The inhibitory effects of the *Moringa oleifera* leaf extract on $CuSO_4$-induced rabbit LDL oxidation were also demonstrated. In HCD group, the lag-time for CD formation and the levels of TBARS formations were significantly different from those of simvastatin or *Moringa oleifera*-treated groups. These results strongly indicate the antioxidant effects of the *Moringa oleifera* leaf extract.

Recent studies demonstrated that atherosclerosis was closely associated with increases in the serum and aortic malondialdehyde (MDA), suggesting of increased levels of oxygen radicals

(Prasad, 2005), and endothelial cell injury (Warren and Ward, 1986), representing a critical initiating event in the development of atherosclerosis. Our study demonstrated that the *Moringa oleifera* leaf extract significantly inhibited the formation of atherosclerotic plaque in internal carotid arteries of rabbits fed with high cholesterol diet. Moreover, the preventive effects on atherosclerotic plaque formation of the extract used in this study are highly comparable to those of simvastatin. Several lines of evidence demonstrated that antioxidants prevent the oxygen radical-induced endothelial cell injury (Siddhuraju and Becker, 2003). From our results, it is clear that the *Moringa oleifera* leaf extract contains antioxidant, hypolipidaemic and antiatherosclerotic activities. Based on the results of our study and other previous studies, it can be suggested that *Moringa oleifera* may serve as a safe and cheap source for the prevention of cardiovascular diseases since this plant has long been used as food and vegetable in many Asian countries and moreover without any reports of toxic effects.

5. Conclusion

Our study clearly demonstrated that water extract of *Moringa oleifera* leaves possesses strong antioxidant activities in both *in vitro* and *ex vivo* experiments. The prevention of atherosclerotic plaque formation in artery as well as the lipid lowering activity of the extract has been shown in rabbit fed with high cholesterol diet. We suggest that *Moringa oleifera* has high therapeutic potential for the prevention of cardiovascular diseases. However, clinical trial in hypercholesterolaemic patients is necessary. In addition, the possibility of phenolic compounds that may contribute to these activities need to be determined experimentally.

Acknowledgements

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