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Inhibition of Human Low-Density Lipoprotein Oxidation In Vitro by Maharishi Ayur-Veda Herbal Mixtures

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SHARMA, H. M., A. N. HANNA, E. M. KAUFFMAN AND H. A. I. NEWMAN. *Inhibition of human low-density lipoprotein oxidation in vitro by Maharishi Ayur-Veda herbal mixtures.* PHARMACOL BIOCHEM BEHAV **43**(4) 1175–1182, 1992. – In this study, we examined the effect of the Maharishi Ayur-Veda herbal mixtures (MAHMs) Maharishi Amrit Kalash-4 and -5 (M-4 and M-5), MA-631, and Maharishi Coffee Substitute (MCS) on low-density lipoprotein (LDL) oxidation and compared the potency of these mixtures to ascorbic acid, α-tocopherol, and probucol. LDL was incubated in 95% air and 5% CO_2, with or without 3 μM Cu^{+2}, in the presence or absence of MAHMs, for 6 or 24 h. In a separate experiment, LDL was incubated as above except MAHMs were added at 0, 1.5, and 3.5 h after incubation started to assess their effect on initiation and propagation of LDL oxidation. Our results demonstrate that MAHMs caused concentration-dependent inhibition of LDL oxidation as assessed by thiobarbituric acid-reactive substances and electrophoretic mobility. The MAHM showed more antioxidant potency in preventing LDL oxidation than ascorbic acid, α-tocopherol, or probucol. Also, MAHMs inhibited both initiation and propagation of cupric ion-catalyzed LDL oxidation. These results suggest the importance of further research on these herbal mixtures in the investigation of atherosclerosis and free radical-induced injury.

LDL oxidation: Initiation and propagation Thiobarbituric acid-reactive substances Maharishi Amrit Kalash-4 Maharishi Amrit Kalash-5 MA-631 Maharishi Coffee Substitute Maharishi Ayur-Veda Vitamin C Vitamin E Probucol Ascorbic acid α-Tocopherol

THERE is increasing evidence that the initiation of atherosclerosis is linked to free radical reactions, including lipid peroxidation (19,51) and oxidative modification of low-density lipoproteins (LDLs) (13,27,30,48). Macrophages take up and degrade native LDL at a low rate without accumulation of cholesterol ester. However, these cells take up and degrade oxidized LDL (LDL-ox) at higher rates through scavenger receptors with the accumulation of cholesterol ester resulting in formation of foam cells, a prominent component of the early events of atherosclerosis (49). LDL-ox is considered crucial for atherogenesis and thus the potential role of antioxidants in prevention of the oxidative modification of LDL acquires great importance. As an example, probucol, an antilipidemic drug with antioxidant properties, causes a significant decrease in the rate of development of atherosclerosis in Watanabe Heritable Hyperlipidemic (WHHL) rabbits (28) despite only a moderate decrease in total cholesterol. This is mainly due to its antioxidant action (3).

Two herbal mixtures known collectively as Maharishi Amrit Kalash (M-4 and M-5) show antioxidant activity (14), as well as inhibiting the growth of mammary tumors in rats (42,43), decreasing platelet aggregation (44), and inhibiting microsomal lipid peroxidation in the rat liver (11). In this investigation, we examined the effects of M-4, M-5, and MA-631, and Maharishi Coffee Substitute (MCS) (which also contain ingredients with antioxidant properties) on the initiation and propagation of the cupric ion-catalyzed oxidation of LDL. Agents that limit LDL oxidation may contribute a significant role in inhibiting the process of atherogenesis.

METHOD

Materials

Malonaldehyde bis(dimethyl acetal) (MDA) and thiobarbituric acid (TBA) were obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). Universal agarose film was obtained from American Scientific Products (Obetz, OH). Ultracentrifuge tubes were purchased from Seton (Sunnyvale, CA). Dialysis membrane (12,000–15,000 Da cutoff) was purchased from Fisher Scientific Co. (Cincinnati, OH). Bovine serum albumin

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(BSA) and L-ascorbic acid, sodium salt (Vitamin C) were purchased from Sigma Chemical Co. (St. Louis, MO). Lyophilized DL-α-tocopherol (Vitamin E) was purchased from Gibco BRL (Gaithersburg, MD). Probucol was a gift from Merrill-Dow (Cincinnati, OH).

The herbal mixtures M-4, M-5, MA-631, and MCS were supplied by Maharishi Ayur-Veda Products International (Lancaster, MA).

Maharishi Ayur-Veda Herbal Mixture Constituents

The ingredients of M-4 (43) and M-5 (44) have been described previously. In brief, the ingredients of M-4 are: raw sugar, ghee (clarified butter), Indian gallnut, Indian gooseberry, dried catkins, Indian pennywort, honey, nutgrass, white sandalwood, butterfly pea, shoeflower, aloewood, licorice, cardamom, cinnamon, cyperus, and turmeric. The ingredients of M-5 are: Gymnema aurentiacum, black musale, heart-leaved moonseed, Sphaerantus indicus, butterfly pea, licorice, Vanda spatulatum, elephant creeper, and Indian wild pepper. On analysis, both M-4 and M-5 have revealed a mixture of low-molecular-weight substances and multiple antioxidants such as α-tocopherol, β-carotene, ascorbate, bioflavonoids, catechin, polyphenol, tannic acid, and riboflavin (42–45). Riboflavin is a constituent of the enzyme glutathione reductase, important in the regeneration of glutathione (an antioxidant peptide). The ingredients of MA-631 are: mineral pitch, Indian bedellium, Himalayan silver fir, dry ginger, catkins, Picrorhiza kurroa, galls, black pepper, Indian kudju, bamboo, Chinese cinnamon, cinnamon, saffron, and cardamom processed in the extracts of Indian trumpet flower, cashmere bark, small caltrops, Aegle marmelos, trumpet flower, Uraria pitch, tinospora, country mallow, licorice, Indian asparagus, winter cherry, Indian kudju, raisins, mycrostylis, globe thistle, cumin seeds, wild cumin, Pluchea lanceolata, Inula racemosa, Baliospermum montanum, major catkins, tellycheri bark, nut grass, Indian gooseberry, Chebulic myrobalans, and Beleric myrobalans. These herbs contain many biologically active ingredients, such as flavonoids, Vitamin C, thiamine, niacin, alkaloids, and rutin. Several of these components have antioxidant properties (2,8,9,20,37,47,52). Also, several of the herbs contained in MA-631 have proven useful in lowering cholesterol levels (52) and treating diabetes mellitus (2,20). MCS contains: clearing nut tree, kasmard, licorice, and winter cherry. These herbs contain glycyrrhizin, flavonoids and their glycosides, coumarin, cinnamic acid derivatives, liquiritin, isoliquiritin, and alkaloids. These ingredients have many biologic activities, including antioxidant activity (1,6,24).

LDL Isolation

Human blood samples (50 ml) were collected in plastic tubes containing EDTA (1 mg/ml), mixed by inversion, and

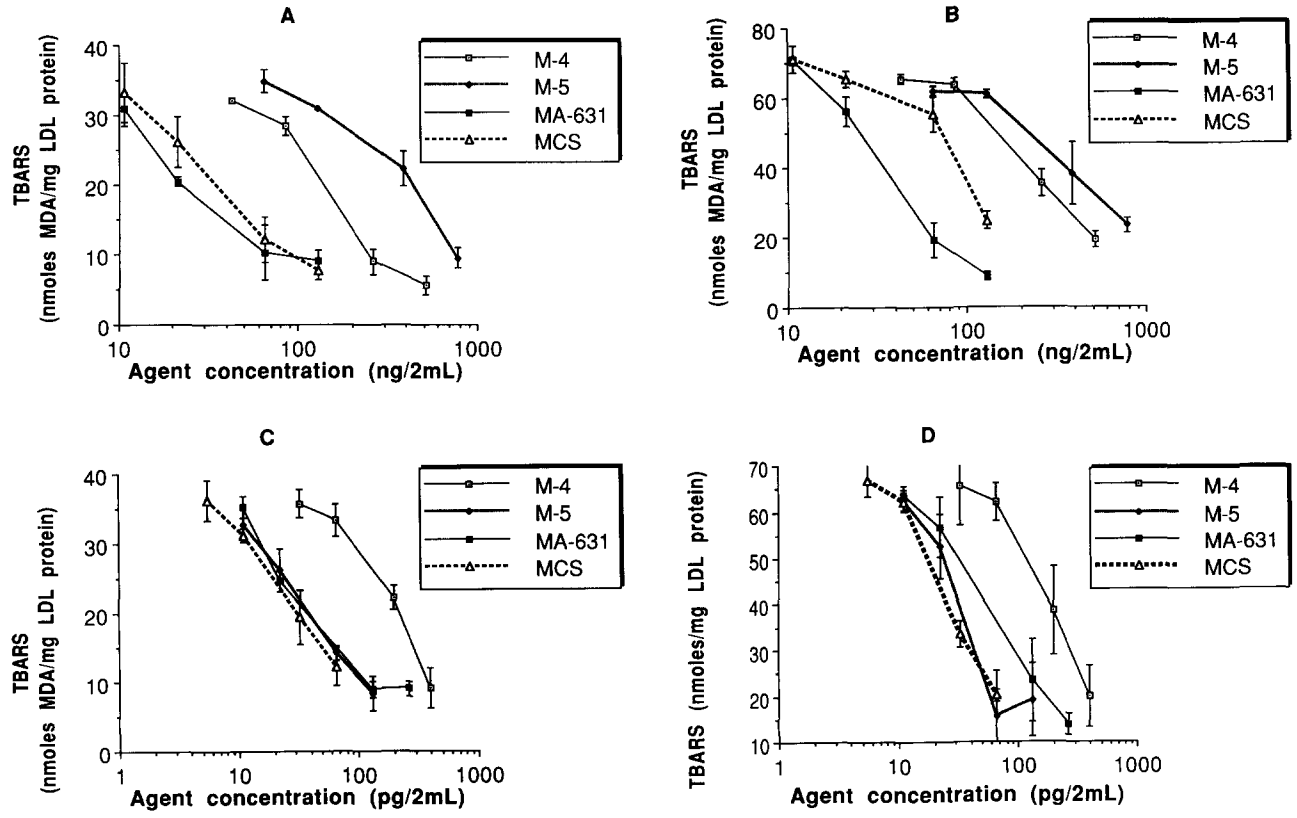

FIG. 1. Concentration-dependent inhibition of Cu^{+2}-catalyzed low-density lipoprotein (LDL) oxidation by Maharishi Ayur-Veda herbal mixtures as assessed by thiobarbituric acid-reactive substances (TBARS) measurement. The conditions for oxidation were as follows: LDL (0.2 mg) was incubated at 37°C in 95% air and 5% CO_2, with 3 μM Cu^{+2}, in the presence of alcoholic extracts of the herbal mixtures for 6 h (A) or 24 h (B). Also LDL was incubated in the presence of aqueous extracts of the herbal mixtures for 6 h (C) or 24 h (D). Values are mean ± SD (n = 3).

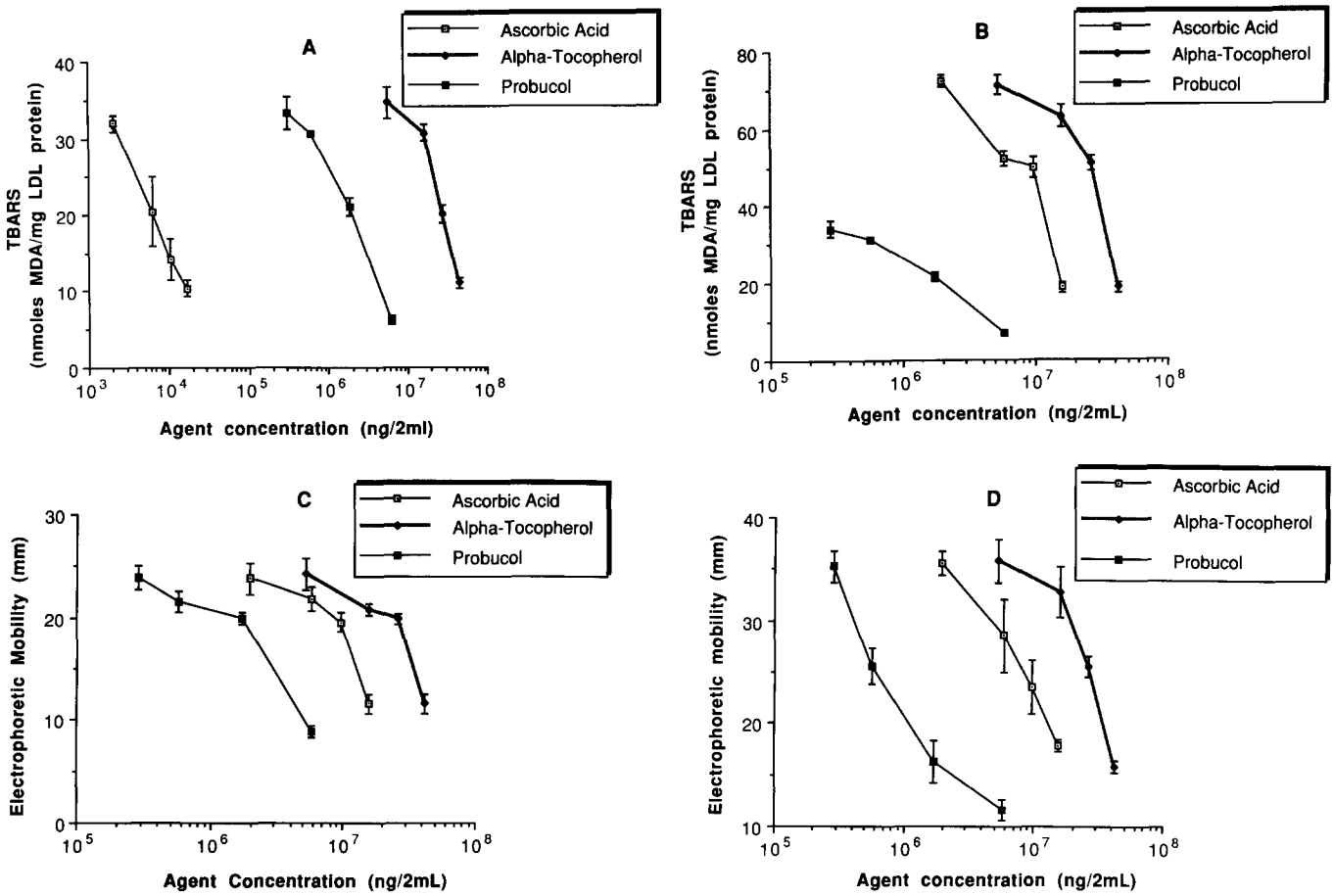

FIG. 2. Concentration-dependent inhibition of low-density lipoprotein (LDL) oxidation by ascorbic acid, α-tocopherol, and probucol as assessed by thiobarbituric acid-reactive substances (TBARS) measurement at 6 h (A) and 24 h (B). Electrophoretic mobility dose-response curves are shown at 6 h (C) and 24 h (D).

kept at 4°C for 3 h. Plasma was separated by centrifugation (2,000× *g*) for 20 min at room temperature. Gentamycin sulfate (1 mg/25 ml) was added to the plasma sample. LDL (d 1.019-1.063) was isolated by sequential isopycnic ultracentrifugations (21). LDL was dialyzed for 16–20 h against 0.01 M phosphate buffer, pH 7.4, containing 0.15 M NaCl and 0.01% EDTA. Protein concentrations of LDL were measured by the Lowry method (29) as modified by Markwell et al. (31). Purity of LDL was assessed by the finding of only one band when electophoregrams were stained for either lipids (fat red 7B) or proteins (amido black).

Agent Preparation

Ascorbic acid and lyophilized α-tocopherol were dissolved in phosphate buffer. Probucol was dissolved in 95% ethanol. Aqueous and alcoholic extracts of the Maharishi Ayur-Veda herbal mixtures (MAHMs) (M-4, M-5, MA-631, and MCS) were prepared by adding 50 mg of these compounds to 25 ml Ham's F-10 media or 95% ethanol. The mixtures were vortexed for 5 min, then centrifuged for 5 min at 2,000× *g*. The supernatant was used for the incubation mixtures. Also, a 20-ml aliquot of the supernatant was lyophilized to calculate the weight of the dissolved substances in relation to the volume used in the incubation mixture.

Incubation Mixtures

LDL (100 μg LDL protein/ml) was incubated in 2 ml Ham's F-10 media, with or without 3 μM Cu^{+2}, in the presence or absence of various concentrations of ascorbic acid, α-tocopherol, probucol, and herbal mixture extracts (aqueous and alcoholic). The mixtures were incubated for 6 or 24 h at 37°C under 95% humidified air and 5% CO_2 (38). The degree of LDL oxidation was assessed by measuring thiobarbituric acid-reactive substances (TBARS) and changes in electrophoretic mobility.

The various agents were added at the end of the incubations to test their interference with the TBARS and electrophoretic mobility assays. None of the agents interfered with these assays. The 95% ethanol used for dissolving probucol and for alcoholic extraction of the herbal compounds did not inhibit the oxidative modification of LDL.

Measurement of TBARS

Aliquots of the incubation mixtures (0.5 ml), containing 50 μg LDL protein, were mixed with 1.5 ml 20% trichloroacetic acid.

Thiobarbituric acid reagent consisting of 1.5 ml 0.67% TBA in 0.05 M NaOH was added with mixing. The mixtures were

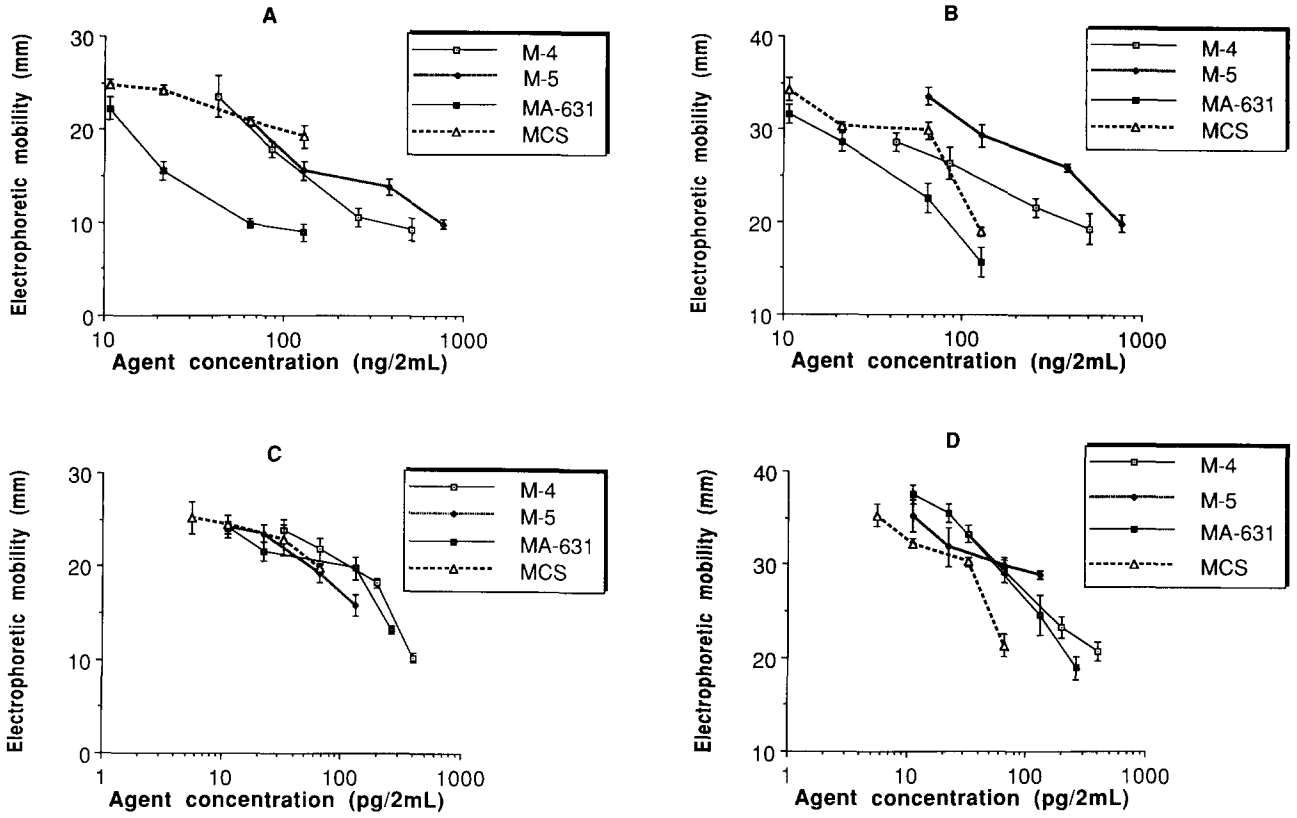

FIG. 3. Concentration-dependent inhibition of low-density lipoprotein (LDL) oxidation by Maharishi Ayur-Veda herbal mixtures as assessed by measurement of electrophoretic mobility. Incubation conditions were the same as in Fig. 1. LDL was incubated in the presence of alcoholic extracts of the herbal mixtures for 6 h (A) or 24 h (B). Also, LDL was incubated in the presence of aqueous extracts of the herbal mixtures for 6 h (C) or 24 h (D). Values are mean ± SD, (n = 3).

TABLE 1

COMPARISON OF IC_{50} (ng/ml) OF DIFFERENT ANTIOXIDANTS ON LDL OXIDATION AFTER 6-h INCUBATION

Agent	TBARS*	Electrophoretic Mobility*
M-4 aqueous	49.0 ± 7.37	48.4 ± 6.79
M-4 alcoholic	0.708 ± 0.222	1.03 ± 0.145
M-5 aqueous	163 ± 53.7	70.4 ± 14.7
M-5 alcoholic	0.132 ± 0.033	0.72 ± 0.31
MA-631 aqueous	10.2 ± 5.51	9.33 ± 1.69
MA-631 alcoholic	0.152 ± 0.055	1.20 ± 0.488
MCS aqueous	11.7 ± 2.16	–
MCS alcoholic	0.132 ± 0.103	0.967 ± 0.737
Ascorbic acid	4.00 ± 0.613 × 10^3	10.5 ± 1.49 × 10^3
α-Tocopherol	19.6 ± 3.90 × 10^3	26.0 ± 4.91 × 10^3
Probucol	1.36 ± 0.658 × 10^3	2.02 ± 0.089 × 10^3

LDL (0.2 mg) was incubated in 95% air and 5% CO_2, with or without 3 μM Cu^{+2}, in the presence or absence of antioxidant agents for 6 h. Values are mean ± SD (n = 3).

*M-4, M-5, MA-631, MCS vs. ascorbic acid, α-Tocopherol, and probucol are significantly different ($p < 0.0001$).

TABLE 2

COMPARISON OF IC_{50} (ng/ml) OF DIFFERENT ANTIOXIDANTS ON LDL OXIDATION AFTER 24-h INCUBATION

Agent	TBARS*	Electrophoretic Mobility*
M-4 aqueous	102 ± 11.2	124 ± 12.6
M-4 alcoholic	0.848 ± 0.387	1.52 ± 0.321
M-5 aqueous	158 ± 70.9	335 ± 55.7
M-5 alcoholic	0.235 ± 0.221	3.653 ± 0.103†
MA-631 aqueous	14.3 ± 5.15	37.3 ± 5.51
MA-631 alcoholic	0.163 ± 0.071	0.988 ± 0.164
MCS aqueous	37.5 ± 8.16	59.2 ± 9.84
MCS alcoholic	0.113 ± 0.028	0.398 ± 0.103
Ascorbic acid	$8.27 \pm 0.678 \times 10^3$	$10.6 \pm 1.70 \times 10^3$
α-Tocopherol	$23.2 \pm 0.924 \times 10^3$	$27.4 \pm 1.46 \times 10^3$
Probucol	453 ± 42.1	$1.45 \pm 0.576 \times 10^3$

Incubation conditions are the same as in Table 1 except incubation was carried out for 24 h. Values are mean ± SD ($n = 3$).

*M-4, M-5, MA-631, MCS vs. ascorbic acid, α-tocopherol, and probucol are significantly different ($p < 0.0001$).

†$n = 2$.

then incubated in an 80–90°C water bath for 45 min, followed by centrifugation at 2,000× g for 10 min. The fluorescence of the supernatant was measured by using excitation and emission wave lengths of 510 and 553 nm, respectively (50).

Measurement of Electrophoretic Mobility

Electrophoresis of LDL was carried out on agarose gel in barbital buffer, pH 8.6, at 90 V for 35 min. The agarose plates were then stained with fat red 7B and excess stain was removed by washing in methanol : water (2 : 1, V/V). The plates were then oven dried for 30 min. Electrophoretic mobility was determined by measuring the distance from the origin to the center of the β-lipoprotein peak on scanned electrophoregrams (35).

Inhibition of Initiation and Propagation of Oxidative Chain Reaction

LDL was isolated as previously described. Incubation mixtures were prepared using 100 μg LDL protein/ml in Ham's F-10 media, with or without 3 μM Cu^{+2}. The incubation was carried out at 37°C in 95% humidified air and 5% CO_2. The various agents were added to the incubation mixtures at 0, 1.5, and 3.5 h in concentrations that totally inhibited the LDL oxidation, as determined from the dose-response curves. The incubation was continued for a total time of 24 h. Incubation mixture controls were carried out in the absence of the various agents, with or without 3 μM Cu^{+2}, for 0, 1.5, 3.5, and 24 h. All mixtures were frozen at −80°C after the appropriate incubation times. The incubation mixtures were subsequently assayed for TBARS and electrophoretic mobility as described above.

Statistical Analyses

The appropriate statistical treatment was an analysis of variance (ANOVA) with repeated measures performed for each of the LDL oxidation parameters to determine if there were any differences among the agents employed. For instances of $p < 0.05$, a posthoc Scheffe's F-test was used to compare each of the agents to probucol (54). All dose responses were tested for significance and correlation by simple regression analysis.

RESULTS

The aqueous and alcoholic extracts of M-4, M-5, MA-631, and MCS, as well as ascorbic acid, α-tocopherol, and probucol, showed a dose-dependent inhibition of cupric ion-catalyzed LDL oxidation after 6- and 24-h incubation. The concentration of TBARS in the incubation mixtures decreased nwith increasing doses of all agents tested (Figs. 1A–1D and 2A and 2B). Accompanying the decrease in TBARS was a

TABLE 3

INHIBITION OF INITIATION OF LDL OXIDATION BY DIFFERENT ANTIOXIDANTS

Conditions	TBARS	Electrophoretic Mobility
LDL alone, frozen at 0 time	7.5	11
LDL + 3 μM Cu^{+2}, incubated for 24 h	43.7	31
LDL + 3 μM Cu^{+2} + 150 ng aqueous extract of M-4, incubated for 24 h	8.6	10
LDL + 3 μM Cu^{+2} + 200 ng aqueous extract of M-5, incubated for 24 h	8.2	10
LDL + 3 μM Cu^{+2} + 150 ng aqueous extract of MA-631, incubated for 24 h	7.9	10
LDL + 3 μM Cu^{+2} + 150 ng aqueous extract of MCS, incubated for 24 h	8.0	11

LDL was incubated under the same conditions as in Table 1. Aqueous extracts of the various agents were added at 0 time and incubation was continued for 24 h. TBARS are expressed as nmoles MDA/mg LDL. Electrophoretic mobility was measured in mm.

reduction in the electrophoretic mobility due to oxidation of LDL (Figs. 2C and 2D and 3A–3D). The data in the dose–response curves were used for calculating the concentration of agents that produced 50% inhibition of LDL oxidation (IC_{50}). Both the aqueous and alcoholic extracts of the herbal compounds proved to be more potent antioxidants than ascorbic acid, α-tocopherol, and probucol with respect to both TBARS and electrophoretic mobility after incubation for 6 and 24 h (Tables 1 and 2). The alcoholic extracts of the herbal compounds were at least 1,000 times more potent than ascorbic acid, α-tocopherol, and probucol after 6-h incubation. After 24-h incubation, the alcoholic extracts of the herbal compounds showed an even larger magnitude of difference. The aqueous extracts of M-4, M-5, MA-631, and MCS were approximately 400, 120, 2,000, and 180 times more potent than α-tocopherol, respectively, after 6-h incubation. This same tendency of potency was also observed when these compounds were compared to ascorbic acid and probucol. Similar differences were also observed after 24 h of incubation.

Incubation of LDL with Cu^{+2} for 1.5 or 3.5 h resulted in initiation of LDL oxidation, as assessed by the increase in TBARS and electrophoretic mobilities compared to those of LDL frozen at 0 time. Also, when the LDL incubation was continued for 24 h there was propagation of LDL oxidation as shown by the continual increase in TBARS and electrophoretic mobilities. The addition of the agents to the incubation mixtures at 0, 1.5, or 3.5 h inhibited the processes of initiation and propagation of LDL oxidation over a 24-h incubation period as assessed by measurement of TBARS and electrophoretic mobility (Tables 3–5).

TABLE 4

INHIBITION OF PROPAGATION OF LDL OXIDATION BY DIFFERENT ANTIOXIDANTS ADDED AFTER 1.5-h INCUBATION

Conditions	TBARS	Electrophoretic Mobility
LDL incubated 1.5 h, then frozen	8.7	11
LDL + 3 μM Cu^{+2}, incubated 1.5 h, then frozen	17.2	12
LDL + 3 μM Cu^{+2} incubated 24 h	43.7	31
LDL + 3 μM Cu^{+2} incubated 1.5 h, then 150 ng M-4 aqueous extract added and incubation continued for 24 h	17.2	9
LDL + 3 μM Cu^{+2} incubated 1.5 h, then 150 ng M-5 aqueous extract added and incubation continued for 24 h	13.2	10
LDL + 3 μM Cu^{+2} incubated 1.5 h, then 150 ng MA-631 aqueous extract added and incubation continued for 24 h	15.4	12
LDL + 3 μM Cu^{+2} incubated 1.5 h, then 150 ng MCS aqueous extract added and incubation continued for 24 h	12.3	10
LDL + 3 μM Cu^{+2} incubated 1.5 h, then 10 μM probucol added and incubation continued for 24 h	15.4	11

Incubation conditions are the same as in Table 1 except the duration of incubation and addition of antioxidant agents is as indicated above. TBARS are expressed as nmoles MDA/mg LDL protein. Electrophoretic mobility was measured in mm.

TABLE 5

INHIBITION OF PROPAGATION OF LDL OXIDATION BY DIFFERENT ANTIOXIDANTS ADDED AFTER 3.5-h INCUBATION

Conditions	TBARS	Electrophoretic Mobility
LDL incubated 3.5 h, then frozen	13.2	11
LDL + 3 μM Cu^{+2}, incubated 3.5 h, then frozen	28.9	15
LDL + 3 μM Cu^{+2} incubated 24 h	43.7	31
LDL + 3 μM Cu^{+2} incubated 3.5 h, then 150 ng M-4 aqueous extract added and incubation continued for 24 h	27.1	15
LDL + 3 μM Cu^{+2} incubated 3.5 h, then 200 ng M-5 aqueous extract added and incubation continued for 24 h	26.6	14
LDL + 3 μM Cu^{+2} incubated 3.5 h, then 150 ng MA-631 aqueous extract added and incubation continued for 24 h	23.0	14
LDL + 3 μM Cu^{+2} incubated 3.5 h, then 150 ng MCS aqueous extract added and incubation continued for 24 h	23.5	15
LDL + 3 μM Cu^{+2} incubated 3.5 h, then 10 μM probucol added and incubation continued for 24 h	26.6	14

Incubation conditions are the same as in Table 1 except the duration of incubation and the addition of antioxidant agents is as indicated above. TBARS are expressed as nmoles MDA/mg LDL protein. Electrophoretic mobility was measured in mm.

DISCUSSION

Reactive oxygen species and free radicals are implicated in a large number of diseases including: inflammatory diseases, degenerative diseases, emphysema, aging, cancer, atherosclerosis, environmental toxicities, reperfusion injury, hyperoxygenation syndrome, and the use of certain chemotherapeutic agents and radiation (41). The use of antioxidants for prevention of damage caused by free radicals thereby assumes great importance. Antioxidants have been found to be advantageous in a large number of diseases (10,32,53). Research done in our laboratory and other investigations have shown that M-4 and M-5 exhibit marked antioxidant (14) and antiplatelet aggregatory activities (44) and prevent rat hepatic microsomal lipid peroxidation (11). M-4 and M-5 have also been shown to prevent 7,12-dimethylbenz(a)anthracene-induced mammary tumors in rats and were effective in treating the fully formed tumors (42,43). In this investigation, we found a marked effectiveness of M-4, M-5, MA-631, and MCS in the inhibition of in vitro LDL oxidation. Both the aqueous and alcoholic extracts of these compounds were found to be more potent than ascorbic acid, α-tocopherol, and probucol in preventing LDL oxidation. The maximum potency of these compounds was seen in the alcoholic extracts, which were at least 1,000 times more potent than ascorbic acid, α-tocopherol, and probucol.

There is rapidly growing evidence that strongly suggests the oxidative modification of LDL plays a key role in the process of atherosclerosis (5,55). LDL can be oxidatively modified by incubation with transition metals such as Cu^{+2} or Fe^{+3} (22) or with arterial wall cells, such as endothelial cells, smooth muscle cells (33), and monocytes (25). Oxidized LDL can occur in vivo as assessed by the presence of autoantibodies that recognize oxidized LDL and other proteins modified by the end product of lipid peroxidation (36). Also, LDL isolated from atherosclerotic lesions of both humans and rabbits has many physical, chemical, and biologic properties similar to oxidized LDL (5,55). LDL-ox is crucial for the atherogenic process because it is cytotoxic (23,34) and causes chemoattraction to monocytes (39), retention of macrophages by inhibiting their mobility (38), increased adhesion of leukocytes to endothelial cells (16), formation of foam cells (49), and inhibition of arterial wall relaxation (46).

It has been reported that Maharishi Amrit Kalash inhibits platelet aggregation (44), an important step in the process of atherosclerotic plaque formation (17). There is increasing interest in the role of diet and nutrition in the pathogenesis of atherosclerosis. Thus, the inverse relationship between ascorbic acid and α-tocopherol levels and susceptibility of LDL to oxidation (12,18,26) has great relevance because prevention of LDL oxidation could diminish the risk of coronary heart disease. Our data demonstrate that the MAHMs used in this investigation inhibit cupric ion-catalyzed oxidation of LDL. Further, we have found that these herbal mixtures inhibit both the initiation and propagation of the oxidative chain reaction of LDL, demonstrating that these herbal mixtures exhibit powerful antioxidant activities. Both the aqueous and alcoholic extracts of the herbal mixtures were more potent antioxidants than ascorbic acid, α-tocopherol, and probucol, likely due to additive or synergistic antioxidant activities. The alcoholic extracts of these mixtures were more potent than the aqueous extracts, perhaps because they contain more lipophilic antioxidants.

Further investigations are needed to demonstrate the potency of the antioxidant activity of these herbal mixtures in vivo. Although the mechanism by which these mixtures inhibit LDL oxidation is not elucidated, inhibition of LDL oxidation may be due to the presence of free radical scavengers or alteration of the structure of LDL particles, rendering them more resistant to oxidation. Analysis of the components of these herbal mixtures has revealed various antioxidants (2,6,8,9,20, 24,37,43,47,52), including various bioflavonoids that have been shown to exhibit antioxidant properties, including the inhibition of lipid peroxidation (4,15,40) and LDL oxidation (7). The flavonoids react rapidly with hydroxyl radicals and are considered of prime importance for radical-scavenging properties (4). It is plausible that these antioxidants are responsible for the prevention of LDL oxidation. The use of these MAHMs needs further exploration in various disease states and toxicities linked to free radical damage, including atherosclerosis and aging.

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