

Evaluation of the Antioxidative and Hypocholesterolemic Effects of Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Agaricomycetes), in Ameliorating Cardiovascular Disease

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ABSTRACT: Oxidative stress (OS) and hypercholesterolemia have been linked with a heightened risk of cardiovascular disease (CVD). Because of the numerous drawbacks of synthetic antioxidants and cholesterol-lowering drugs, natural antioxidative and hypocholesterolemic agents are of immense importance. This study was designed to determine both the OS-attenuating and cholesterol-lowering capacities of the hot water extract (HWE) and of five solvent-solvent-partitioned fractions of *Ganoderma lucidum*. *In vitro* antioxidative performance of *G. lucidum* HWE and fractions was measured through DPPH free radical scavenging, Folin-Ciocalteu assay, lipid peroxidation inhibition, and human low-density lipoprotein (LDL) oxidation inhibition. *In vivo* antioxidative performance of *G. lucidum* was assessed by measuring the plasma and liver antioxidative enzymatic activities (catalase, glutathione peroxidase, and superoxide dismutase) in *G. lucidum* HWE-fed rats. In the CVD tests, the HWE at 200 mg/kg b.w. lowered plasma levels of total cholesterol, triacylglycerol, and LDL cholesterol and increased high-density lipoprotein cholesterol. The current findings indicate the therapeutic potentiality of *G. lucidum* as an OS-attenuating and hypocholesterolemic agent en route to withstanding CVD complications.

KEY WORDS: *Ganoderma lucidum*, body weight, lipid peroxidation, low-density lipoprotein, oxidative stress, toxicity, medicinal mushrooms

ABBREVIATIONS: AIP, atherogenic index of plasma; ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; C, control rats; CE, *G. lucidum* HWE-fed control rats; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; GPx, glutathione peroxidase; H, hypercholesterolemic rats; HDL-C, high-density lipoprotein cholesterol; HE, *G. lucidum* HWE-fed hypercholesterolemic rats; HMG-CoA, 3-hydroxy 3-methyl glutaryl coenzyme A; HMGR, 3-hydroxy 3-methyl glutaryl coenzyme A reductase; LDL-C, low-density lipoprotein cholesterol; MDA, malondialdehyde; OS, oxidative stress; ROS, reactive oxygen species; SOD, superoxide dismutase; TC, total cholesterol; TG, triacylglycerol

I. INTRODUCTION

Under normal physiological conditions, reactive oxygen species (ROS) and reactive nitrogen species are generated as a part of cellular metabolic, signaling, and defensive actions.¹ Because they are highly reactive, they undergo reactions with other reactive anions and/or cations and turn into neutral molecules, or the bodily systems neutralize, remove, or attenuate them through different mechanisms.² Thus, these reactive species have very short half-lives.² However, when the level of their generation outnumbers their dismissal through enzymatic or nonenzymatic processes, the state of oxidative stress (OS) emanates. Persistent OS mediates structural and functional alteration of biomolecules (carbohydrates, proteins, lipids, nucleic acids) and poses a threat to normal functioning of the tissues and organs, resulting in numerous pathophysiological consequences such as cardiovascular diseases (CVDs), diabetes, aging, and neurodegenerative diseases such as Alzheimer's disease.^{2,3}

Evolutionarily, cells have adapted themselves to cope with the constant exposure to ROS using multiple defense strategies.⁴ Cells thwart OS through physical defense involving stabilization of plasma membrane

and steric hindrance. The antioxidative enzymatic defense system includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which counterbalance the production of ROS.² SOD first converts the superoxide into relatively less harmful hydrogen peroxide; later on, CAT and GPx turn hydrogen peroxide into water.^{2,5} Also, nonenzymatic intracellular antioxidants act directly by scavenging free radicals or by breaking the free radical-generating chain reactions. This type of antioxidant includes glutathione, flavonoids, pyruvate, vitamin C (ascorbic acid), vitamin E (α tocopherol), and carotenoids.⁵ Cells synthesize a trivial amount of the direct-acting antioxidants, and most of them must be supplied through the diet for maintenance of normal cellular functioning against OS.⁵ Thus, the supply of the exogenous antioxidants (natural or synthetic) seemed promising as a preventive therapeutic stratagem against ROS-mediated pathophysiology. However, both natural and synthetic antioxidants are available; in this regard, the second form of antioxidant causes numerous side effects.⁶⁻⁹ Thus, the demand for natural, dietary antioxidants has gained momentum.⁵

Hypercholesterolemia refers to the elevated level of cholesterol in the blood. It is a form of hyperlipidemia (elevated lipid level in blood) also ascribed to dyslipidemia.¹⁰ Cholesterol metabolism occurs in the liver. The rate-limiting step of cholesterol biosynthesis is mediated by 3-hydroxy 3-methyl glutaryl coenzyme A (HMG-CoA) reductase (HMGR). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice has suggested lifestyle and dietary modification as the first line of treatment and pharmacological intervention through lipid-lowering drugs as the second line of treatment against hypercholesterolemia.¹¹ Lifestyle modification involves losing weight, consuming a diet containing less cholesterol and higher unsaturated fatty acids, giving up cigarette smoking and drinking alcohol, increasing intake of unsaturated fatty acids such as docosahexaenoic acid, and engaging in regular physical exercise.¹²⁻¹⁴ However, a combination of both lifestyle modification and pharmacological treatment may be more realistic.¹⁵

Mushrooms are among the earliest natural agents used by humans.¹⁶⁻¹⁸ The lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (W.Curt.:Fr.) P. Karst. (Ganodermataceae, Agaricomycetes), has been highly appreciated due to its numerous medicinal properties.¹⁹⁻²³ Although different studies have demonstrated the antioxidative and hypocholesterolemic effects of *G. lucidum*, studies incorporating both aspects are scarce. Thus, in the present study, we sought to determine the *in vitro* antioxidative performance of the solvent-solvent partitioned fractions and of the hot water extract (HWE) of *G. lucidum*, followed by an assessment of the HWE's *in vivo* antioxidative and hypocholesterolemic potentiality.

II. MATERIALS AND METHODS

A. Mushroom Material and Preparation of Solvent-Solvent-Partitioned Fractions and HWE of *G. lucidum*

Fruiting bodies of *G. lucidum* were purchased from a mushroom farm in Tanjung Sepat, Selangor, Malaysia. The mushroom was identified and authenticated by experts via DNA sequencing by the University of Malaya Mushroom Research Centre herbarium. A voucher specimen was deposited in the University of Malaya herbarium (KLU-M1233). We prepared the solvent-solvent partitioned fractions and HWE of *G. lucidum* using methanol:dichloromethane, dichloromethane, hexane, ethyl acetate, and water.²⁴

B. *In Vitro* Studies

We measured the *in vitro* antioxidative effects of all the solvent-solvent partitioned fractions and of the HWE of *G. lucidum* following standard tests. Our *in vitro* tests included DPPH free-radical scavenging effects, total polyphenol content through the Folin-Ciocalteu assay, lipid peroxidation inhibitory test, and

human low-density lipoprotein (LDL) oxidation inhibitory test.^{25–28} The inhibitory effects of the fractions and of the *G. lucidum* HWE were also tested.²⁹

C. In Vivo Studies

1. Animals and Maintenance

Sixty Wistar male rats (weight range, 120 ± 5 g) were randomly divided into four groups: 1) control (C), 2) *G. lucidum* HWE-fed control (CE), 3) hypercholesterolemic (H), and 4) *G. lucidum* HWE-fed hypercholesterolemic (HE) rats. *In vivo* antioxidative and hypocholesterolemic studies of each of the four groups were performed. Hypercholesterolemia in the H rats was evoked by adding 1% cholesterol and 1% cholic acid (for intestinal better absorption of cholesterol) to the basal diet. The composition of the basal diet was as follows (in g/100 g): 50 g wheat flour, 19 g wheat bran, 11.25 g rice polishings, 8 g casein, 10 g egg white, 1 g soybean oil, 0.5 g table salt, 0.125 g vitamin mixture, and 0.125 g mineral mixture. Egg white was replaced with 5% dried mushroom powder for diets fed to the mushroom-fed groups (NC + MS and HC + MS). The composition of the vitamin mixture in the diet was as follows (in g/100 g vitamin mixture): 9.5×10^{-4} g retinyl acetate, 1.2×10^{-3} g cholecalciferol, 0.05 g α -tocopherol acetate, 2.4 g thiamine HCl, 2.4 g riboflavin, 12.0 g nicotinic acid, 9.6 g D-calcium pantothenate, 1.2 g pyridoxine HCl, 9.5×10^{-2} g folic acid, 0.25 g vitamin K, 9.5×10^{-3} g cyanocobalamine, 47.95 g inositol, and 24.0 g ascorbic acid. The composition of the mineral mixture added to the diet was as follows (in g/100 g vitamin mixture): 28.5 g calcium gluconate, 17.3 g K_2HPO_4 , 26.0 g $CaCO_3$, 12.6 g $MgSO_4$, 12.6 g KCl, 0.06 g $CuSO_4$, 0.30 g $FeSO_4$, 0.55 g $MnSO_4$, 2.5×10^{-4} g NaF, 9.0×10^{-4} g KI, 3.0×10^{-4} g Na-molybdate, 3.0×10^{-4} g SeO_2 , and 1.5×10^{-3} g $CrSO_2$. Rats were fed for 8 weeks. The extract-fed groups (CE and HE) received 200 mg/kg b.w. *G. lucidum* HWE as the treatment from day 1 until the end of the experiment. Animals were housed on a 12-hour day/night cycle at 25 ± 2 °C. Feeding, drinking, and treatment were stopped 24 hours before sacrifice. All of the experimental protocols were approved by the ethical permission committee [University of Malaya Institutional Animal Care and Use Committee; Ethics Reference No. ISB/25/04/2013/NA (R)].

2. In Vivo Antioxidative, Hypocholesterolemic, and Organ Function Tests

For *in vivo* antioxidative studies, activity of the plasma and/or liver parameters of antioxidative enzymes CAT, GPx, and SOD of the extract-fed and control animals were measured utilizing commercially available kits (item nos. 707002 for CAT, 703102 for GPx, and 706002 for SOD; Cayman Chemical, Ann Arbor, MI) following the manufacturer's instructions.^{30–33} The atherogenic index of plasma (AIP), the logarithmically transformed ratio of molar concentrations of triglycerides to high-density lipoprotein cholesterol (HDL-C), was also measured.³⁴ We calculated the ratio of plasma LDL-C to HDL-C for measuring the LDL:HDL ratio. Using commercially available kits (Sigma-Aldrich, St. Louis, MO), the activity of aspartate aminotransferase (AST)/glutamate oxalate transaminase (GOT), alanine aminotransferase (ALT)/glutamate pyruvate transaminase (GPT), bilirubin, creatinine, and urea levels was determined.^{35–39}

D. Statistical Analyses

All the experiments were conducted in triplicate, and we present data as the mean \pm SEM. We used SPSS version 16 software, and we performed analysis of variance followed by *post hoc* Tukey's honestly significant difference test at the 95% level.

III. RESULTS AND DISCUSSION

A. *In Vitro* Studies

Among six fractions, the HWE of *G. lucidum* mostly (55.13%, significant at $P \leq 0.05$) scavenged the DPPH free radical (Table 1). The HWE of *G. lucidum* possessed the best reducing capacity; it had the highest content (in milligrams) of gallic acid equivalents per gram of extract (Table 1). In the present study, inhibitory performance of the mushroom extracts upon lipid peroxidation of the buffered egg yolk was assessed through the measurement of thiobarbituric acid reactive substances. Lipophilic biocomponents present in the nonpolar hexane may have inhibited lipid peroxidation and LDL oxidation in the present experiment. Compared to others, the hexane and the HWE of *G. lucidum* showed better inhibitory effects toward HMGR activity (Table 2). The presence of statin in the *G. lucidum* fractions was not investigated in the present study; thus, we cannot confirm that natural statins were present in the respective fractions. However, ergosterol was present in the hexane fraction, which might be regarded as an inhibitor of HMGR activity.⁴⁰⁻⁴²

B. *In Vivo* Studies

Compared with the control rats, hypercholesterolemic rats had lower levels of plasma and liver antioxidant enzymatic (CAT, GPX, and SOD) activities (Tables 3 and 4). Feeding *G. lucidum* HWE increased enzymatic antioxidative defense of the rats (Tables 3 and 4). During 8 weeks of observation, the hypercholesterolemic (H) rats gained the maximum weight, and their rate of weight gain surpassed that of the others (ranging from 140 ± 5 g up to 280 ± 2 g) (Fig. 1). However, feeding *G. lucidum* HWE resulted in decreased weight gain in the HE rats as time passed. Feeding a hypercholesterolemic diet to the rats resulted in increased plasma triacylglycerol (TG) levels (Table 5). The hypercholesterolemic (H) rats had a 1.58 times higher plasma TG level than the control (C) rats, indicating the increased atherogenic propensity of the H rats. Feeding of *G. lucidum* HWE lowered the plasma TG level in both the control (15%) and the

TABLE 1: *In Vitro* Antioxidant Potentiality

Parameter	<i>Ganoderma lucidum</i> Fractions and Extract						Standard
	MD	HEX	DCM	EA	AQ	HWE	
DPPH free radical scavenging (%)	42.36 ± 1.44	23.03 ± 12	24.76 ± 0.82	25.54 ± 1.27	22.85 ± 1.08	55.13 ± 0.48	75.15 ± 0.48
Total polyphenol content	51.36 ± 0.9	40.45 ± 0.7	52.42 ± 0.7	75.3 ± 1.46	43.3 ± 0.95	81.06 ± 0.70	95.91 ± 1.64
Lipid peroxidation inhibition	43.55 ± 0.64	58.56 ± 0.37	55.47 ± 1.11	35.60 ± 0.98	43.3 ± 0.85	45.58 ± 0.98	80.54 ± 2.43
LDL oxidation inhibition MDA (µM/mg LDL)	21.34 ± 1.26	14.65 ± 0.72	17.58 ± 1.26	20.93 ± 2.61	25.10 ± 2.51	23.01 ± 1.92	10.46 ± 2.61

Data are expressed as mean ± SE of triplicate values. Standards used were quercetin (for free radical scavenging test), gallic acid (for total polyphenolic test), α -tocopherol (for lipid peroxidation), and linoleic acid (for LDL oxidation test). AQ, aqueous fraction; DCM, dichloromethane; EA, ethylacetate; HEX, hexane; HWE, hot water extract of *G. lucidum* at 1 mg/mL; MD, methanol:dichloromethane.

TABLE 2: HMGR Inhibitory Effect of *Ganoderma lucidum* HWE

<i>G. lucidum</i> Fraction, Extract and Standard	Inhibition of HMGR Activity (%)
MD	29.09 ± 0.91
HEX	53.33 ± 1.89
DCM	48.18 ± 0.90
EA	45.45 ± 2.72
AQ	30.30 ± 1.89
HWE	52.12 ± 1.3
Pravastatin	75.76 ± 2.7

Data are expressed as mean ± SE of triplicate values. Pravastatin was used as the standard. AQ, aqueous fraction; DCM, dichloromethane; EA, ethylacetate; HEX, hexane; HMGR, HMG-CoA reductase; HWE, hot water extract of *G. lucidum* at 1 mg/mL; MD, methanol:dichloromethane.

hypercholesterolemic (13%) groups (Table 5). The TG-lowering effect of *G. lucidum* HWE was statistically significant in each group compared to their respective controls (Table 5). TG regulates lipoprotein interactions and thus plays an important role in maintaining lipid homeostasis. Its own level also varies depending on the lipoproteins, especially LDL-C. Increased TG levels in the hypercholesterolemic rats (Table 5) might be due to the decreased clearance of TG due to lower lipoprotein lipase activity or to increased deposition of LDL-C.⁴³

TABLE 3: Plasma Antioxidant Enzymatic Activity

Plasma Antioxidant Enzyme Level (nmol/mL)	C	CE	H	HE
Catalase	9.83 ± 0.10	14.66 ± 0.30	7.34 ± 0.30	11.78 ± 0.23
Glutathione peroxidase	29.53 ± 0.70	42.23 ± 0.22	26.03 ± 0.5	32.37 ± 0.34
Superoxide dismutase	59.96 ± 0.80	67.42 ± 0.42	48.20 ± 0.6	50.27 ± 0.40

Data are expressed as mean ± SE of triplicate values. C, control group; CE, *Ganoderma lucidum* HWE-fed control group; H, hypercholesterolemic group; HE, *G. lucidum* HWE-fed H group.

TABLE 4: Liver Antioxidant Enzymatic Activity

Liver Antioxidant Enzyme Level (nmol/mL)	C	CE	H	HE
Catalase	59.64 ± 0.53	67.88 ± 0.37	46.55 ± 0.52	52.4 ± 0.54
Glutathione peroxidase	55.80 ± 0.32	59.93 ± 0.12	49.26 ± 0.68	54.15 ± 0.37
Superoxide dismutase	85.46 ± 0.53	90.55 ± 0.16	78.92 ± 1.19	82.34 ± 0.54

Data are expressed as mean ± SE of triplicate values. C, control group; CE, *Ganoderma lucidum* HWE-fed control group; H, hypercholesterolemic group; HE, *G. lucidum* HWE-fed H group.

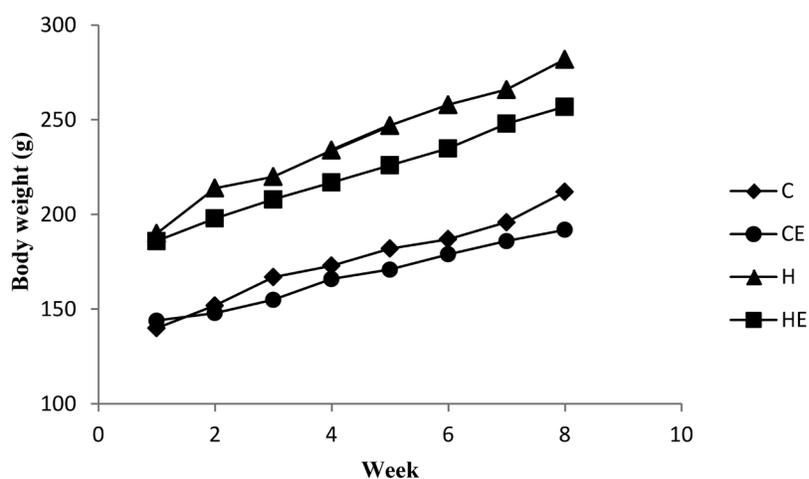


FIG. 1: Effect of *Ganoderma lucidum* HWE feeding (200 mg/kg b.w.) upon body weight (g) of the rats. C, control group; CE, *G. lucidum* HWE-fed control group; H, hypercholesterolemic group; HE, *G. lucidum* HWE-fed control group.

Compared with the C rats, the H rats showed 1.5 times increased plasma total cholesterol (TC) levels (Table 4). The *G. lucidum* HWE treatment lowered the plasma TC level significantly ($P \leq 0.05$) in all the rat groups (Table 5). The TC-lowering effect of *G. lucidum* might be mediated by several mechanisms. Importantly, the ganoderic acids share the structural features of the active site of HMG-CoA and might bind in the active site of HMGR that contributed its competitive inhibition of HMGR and lowered cholesterol biosynthesis. Also, gano-polyphenolics might bind with bile acids and lower solubility of cholesterol in micelles.⁴⁴ On the other hand, significantly increased plasma HDL-C level ($P \leq 0.05$) in the *G. lucidum* HWE-fed rats indicates increased clearance of TC from the peripheral tissue to the liver for excretion that points to a CVD-ameliorating effect of *G. lucidum* HWE. *G. lucidum* HWE supplementation caused significant lowering effect upon the plasma level of both LDL-C and VLDL-C in all the rat groups (Table 5). Mechanistically, *G. lucidum* HWE induced increased LDL receptors in the rat hepatocytes, which might contribute to the LDL-lowering effect that resulted in lowered secretion of LDL in the rat plasma. Considering the AIP, the H rats had higher AIP than the C rats, and *G. lucidum* HWE treatment decreased AIP (Table 4). The increased AIP of the hypercholesterolemic rats observed in the present study (Table 5)

TABLE 5: Cholesterol-Lowering Effect of *Ganoderma lucidum* Hot Water Extract upon Rat Plasma Parameters

Plasma Parameter (mg/dL)	C	CE	H	HE
TG	122.73 ± 0.63	103.93 ± 0.85	192.0 ± 0.880	167.33 ± 0.90
TC	90.6 ± 0.74	81.9 ± 0.91	135.2 ± 0.84	109.4 ± 1.0
HDL-C	29.06 ± 0.42	36.47 ± 0.55	26.94 ± 0.58	33.33 ± 0.40
LDL-C	36.97 ± 0.58	24.68 ± 0.10	69.86 ± 0.90	42.60 ± 1.34
VLDL-C	24.54 ± 0.13	20.78 ± 0.17	38.40 ± 0.18	33.47 ± 0.18
AIP	2.12 ± 0.04	1.25 ± 0.04	4.05 ± 0.10	2.29 ± 0.05
LDL/HDL	1.28 ± 0.02	0.68 ± 0.04	2.6 ± 0.08	1.29 ± 0.05

Data are expressed as mean ± SE of triplicate values. C, control; CE, *G. lucidum* HWE-fed control; H, hypercholesterolemic; HE, *G. lucidum* HWE-fed hypercholesterolemic rats.

TABLE 6: Organ Function/Toxicity Tests

Organ Function Tests	C	CE	H	HE
SGOT/AST (U/L)	50.8 ± 0.42	52.33 ± 0.44	74.67 ± 0.73	63.33 ± 0.40
SGPT/ALT (U/L)	19.93 ± 0.50	21.66 ± 0.37	28.67 ± 0.5	22.87 ± 0.276
ALP (U/L)	65.73 ± 0.63	60.06 ± 0.52	106.07 ± 0.51	94.06 ± 0.61
Bilirubin (mg/dL)	0.21 ± 0.006	0.22 ± 0.005	0.53 ± 0.01	0.33 ± 0.005
Creatinine (mg/dL)	0.25 ± 0.005	0.27 ± 0.023	0.66 ± 0.01	0.53 ± 0.01
Urea (mg/dL)	15.80 ± 0.48	15.67 ± 0.31	24.80 ± 0.44	19.87 ± 0.73

Data are expressed as mean ± SE of triplicate values. C, control; CE, *Ganoderma lucidum* HWE-fed control; H, hypercholesterolemic; HE, *G. lucidum* HWE-fed hypercholesterolemic rats.

might have been caused by the increased level of the atherogenic lipoprotein fractions (LDL-C) associated with hyperlipidemia.^{45,46} The LDL/HDL ratio is another indicator of hyperlipidemia that led to physiological complications, including atherosclerosis.⁴⁷ A higher HDL level, lower LDL level, and lowered LDL/HDL ratio were expected for the hyperlipidemia-modulating agents.⁴⁷ *G. lucidum* HWE feeding was able to lower the LDL/HDL ratio in every rat group (Table 5).

Feeding *G. lucidum* HWE at the dosage of 200 mg/kg b.w. did not cause any visible adverse effect or death of the experimental animals. Compared with the control and the *G. lucidum* HWE-fed rats, the hypercholesterolemic rats were detected with elevated levels of serum AST/GOT, ALT/GPT, and ALP enzymes in the present study (Table 6). These are the enzymes of diagnostic importance that increase in the blood, as they leak out of the damaged cell membranes following CVD complications. Thus, cholesterol affected the plasma membrane integrity and permeability of the hypercholesterolemic rats. In a similar fashion, significantly ($P \leq 0.05$) increased bilirubin, creatinine, and urea levels in the hypercholesterolemic rats also indicated the cholesterol-mediated perturbed metabolism of the respective animals (Table 6). Thus, *G. lucidum* HWE was safe and nontoxic to the experimental animals.

ACKNOWLEDGMENTS

This research was performed with financial aid from the University of Malaya (UMRG Funding RP014D-13AFR, PPP Grant No. PG109-2013B, and HIR-MOHE Research Grant No. F000002-21001). M.A.R. is grateful for the fellowship supported by the Bright Sparks Unit of the University of Malaya.

REFERENCES

1. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature*. 2000;408(6809):239–47.
2. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44–84.
3. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. *Clin Chem*. 2006;52(4):601–23.
4. Kedare SB, Singh R. Genesis and development of DPPH method of antioxidant assay. *J Food Sci Technol*. 2011;48(4):412–22.
5. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. *Pharmacogn Rev*. 2010;4(8):118–26.
6. Botterweck A, Verhagen H, Goldbohm R, Kleinjans J, Van den Brandt P. Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands cohort study. *Food Chem Toxicol*. 2000;38(7):599–605.

7. Kahl R. Synthetic antioxidants: biochemical actions and interference with radiation, toxic compounds, chemical mutagens and chemical carcinogens. *Toxicology*. 1984;33(3):185–228.
8. Umemura T, Kodama Y, Hioki K, Inoue T, Nomura T, Kurokawa Y. Butylhydroxytoluene (BHT) increases susceptibility of transgenic rasH2 mice to lung carcinogenesis. *J Cancer Res Clin Oncol*. 2001;127(10):583–90.
9. Shahidi F, Zhong Y. Antioxidants: regulatory status. In: Bailey's industrial oil and fat products. 6th ed. Hoboken (NJ): Wiley; 2005.
10. Durrington P. Dyslipidaemia. *Lancet*. 2003;362(9385):717–31.
11. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syv anne M, Scholte op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F; European Association for Cardiovascular Prevention & Rehabilitation (EACPR); ESC Committee for Practice Guidelines (CPG). European guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). *Eur Heart J*. 2012;33(13):1635–701.
12. Brown T, Avenell A, Edmunds LD, Moore H, Whittaker V, Avery L. Systematic review of long-term lifestyle interventions to prevent weight gain and morbidity in adults. *Obes Rev*. 2009;10(6):627–38.
13. Klein S, Burke LE, Bray GA, Blair S, Allison DB, Pi-Sunyer X, Hong Y, Eckel RH. Clinical implications of obesity with specific focus on cardiovascular disease a statement for professionals from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: Endorsed by the American College of Cardiology Foundation. *Circulation*. 2004;110(18):2952–67.
14. Galani C, Schneider H. Prevention and treatment of obesity with lifestyle interventions: review and meta-analysis. *Int J Public Health*. 2007;52(6):348–59.
15. Last AR, Ference JD, Falleroni J. Pharmacologic treatment of hyperlipidemia. *Am Fam Physician*. 2011;84(5):551.
16. Wasser SP. Medicinal mushroom science: history, current status, future trends, and unsolved problems. *Int J Med Mushrooms*. 2010;12(1):1–16.
17. Chang ST, Wasser SP. The role of culinary-medicinal mushrooms on human welfare with pyramid model for human health. *Int J Med Mushrooms*. 2012;14(2):95–134.
18. Wasser SP. Medicinal mushrooms in human clinical studies. Part I. Anticancer, oncoimmunological, and immunomodulatory activities: a review. *Int J Med Mushrooms*. 2017;19(4):279–317.
19. Batra P, Sharma AK, Khajuria R. Probing Lingzhi or Reishi medicinal mushroom *Ganoderma lucidum* (higher Basidiomycetes): a bitter mushroom with amazing health benefits. *Int J Med Mushrooms*. 2013;15(2):127–43.
20. Cizmarikova M. The efficacy and toxicity of using the lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (Agaricomycetes), and its products in chemotherapy (review). *Int J Med Mushrooms*. 2017;19(10):861–77.
21. Kirar V, Nehra S, Mishra J, Rakhee, Saraswat D, Misra K. Lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (Agaricomycetes), as a cardioprotectant in an oxygen-deficient environment. *Int J Med Mushrooms*. 2017;19(11):1009–21.
22. Zhou S, Gao Y, Chan E. Clinical trials for medicinal mushrooms: experience with *Ganoderma lucidum* (W.Curt.:Fr.) Lloyd (Lingzhi mushroom). *Int J Med Mushrooms*. 2005;7(1–2):111–8.
23. Martin KR. Both common and specialty mushrooms inhibit adhesion molecule expression and in vitro binding of monocytes to human aortic endothelial cells in a pro-inflammatory environment. *Nutr J*. 2010;9:29.
24. Abidin MHZ, Abdullah N, Abidin N. Protective effect of antioxidant extracts from grey oyster mushroom, *Pleurotus pulmonarius* (Agaricomycetes), against human low-density lipoprotein oxidation and aortic endothelial cell damage. *Int J Med Mushrooms*. 2016;18(2):109–21.
25. Abdullah N, Ismail SM, Aminudin N, Shuib AS, Lau BF. Evaluation of selected culinary-medicinal mushrooms for antioxidant and ACE inhibitory activities. *Evid Based Complement Alternat Med*. 2012;2012:464238.
26. Slinkard K, Singleton, VL. Total phenol analysis: automation and comparison with manual methods. *Am J Enol Viticul*. 1977;28(1):49–55.
27. Daker M, Abdullah N, Vikineswary S, Goh PC, Kuppusamy UR. Antioxidant from maize and maize fermented by *Marasmiellus* sp. as stabiliser of lipid-rich foods. *Food Chem*. 2008;107(3):1092–98.
28. Rahman MA, Abdullah N, Aminudin N. Inhibitory effect on in vitro LDL oxidation and HMG Co-A reductase activity of the liquid-liquid partitioned fractions of *Hericium erinaceus* (Bull.) Persoon (lion's mane mushroom). *Biomed Res Int*. 2014;2014:828149.
29. Gholamhoseinian A, Shahouzehi B, Sharifi-Far F. Inhibitory activity of some plant methanol extracts on 3-hydroxy-3-methylglutaryl coenzyme a reductase. *Int J Pharmacol*. 2010;6(5):705–11.
30. Werner M, Gabrielson DG, Eastman J. Ultramicro determination of serum triglycerides by bioluminescent assay. *Clin Chem*. 1981;27(2):268–71.
31. Allain CC, Poon LS, Chan CSG, Richmond WFPC, Fu PC. Enzymatic determination of total serum cholesterol. *1974;20(4):470–5*.

32. Izzo C, Grillo F, Murador E. Improved method for determination of high-density-lipoprotein cholesterol I. Isolation of high-density lipoproteins by use of polyethylene glycol 6000. *Clin Chem.* 1981;27(3):371–4.
33. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18(6):499–502.
34. Harnafi H, Caid HS, el Houda B, Nour A, Mohammed AS. Hypolipemic activity of polyphenol-rich extracts from *Ocimum basilicum* in Triton WR-1339-induced hyperlipidemic mice. *Food Chem.* 2008;108(1):205–12.
35. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Path.* 1957;28(1):56–63.
36. Tietz NW, Burtis CA, Duncan P, Ervin K, Petitclerc CJ, Rinker AD, Shuey D, Zygowicz ER. A reference method for measurement of alkaline phosphatase activity in human serum. *Clin Chem.* 1983;29(5):751–61.
37. Mori L. Modified Jendrassik-Grof method for bilirubins adapted to the Abbott bichromatic analyzer. *Clin Chem.* 1978;24(10):1841–5.
38. Bonsnes RW, Taussky HH. On the colorimetric determination of creatinine by the Jaffe reaction. *J Biol Chem.* 1945;158(3):581–91.
39. Jung D, Biggs H, Erikson J, Ledyard PU. New colorimetric reaction for end-point, continuous-flow, and kinetic measurement of urea. *Clin Chem.* 1975;21(8):1136–40.
40. Gil-Ramírez A, Clavijo C, Palanisamy M, Ruiz-Rodríguez A, Navarro-Rubio M, Pérez M. Screening of edible mushrooms and extraction by pressurized water (PWE) of 3-hydroxy-3-methyl-glutaryl CoA reductase inhibitors. *J Func Food.* 2013;5(1):244–50.
41. Gil-Ramírez A, Clavijo C, Palanisamy M, Soler-Rivas C, Ruiz-Rodríguez A, Marín FR. Edible mushrooms as potential sources of new hypocholesterolemic compounds. Proceedings of the 7th International Conference on Mushroom Biology and Mushroom Products. Arcachon, France, 2011.
42. Gil-Ramírez A, Clavijo C, Palanisamy M, Ruiz-Rodríguez A, Navarro-Rubio M, Pérez M. Study on the 3-hydroxy-3-methyl-glutaryl CoA reductase inhibitory properties of *Agaricus bisporus* and extraction of bioactive fractions using pressurised solvent technologies. *J Sci Food Agril.* 2013;93(11):2789–96.
43. Nofer J-R, Kehrel B, Fobker M, Levkau B, Assmann G, von Eckardstein A. HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atheroscler.* 2002;161(1):1–16.
44. Ngamukote S, Mäkynen K, Thilawech T, Adisakwattana S. Cholesterol-lowering activity of the major polyphenols in grape seed. *Molecules.* 2011;16(6):5054–61.
45. Dobiasova M. AIP--atherogenic index of plasma as a significant predictor of cardiovascular risk: from research to practice. *Vnitri Lekarstvi.* 2006;52(1):64–71.
46. Dobiášová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER HDL). *Clin Biochem.* 2001;34(7):583–8.
47. Granholm A-C, Bimonte-Nelson HA, Moore AB, Nelson ME, Freeman LR, Sambamurti K. Effects of a saturated fat and high cholesterol diet on memory and hippocampal morphology in the middle-aged rat. *J Alz Dis.* 2008;14(2):133–45.

